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INTERACTION OF EXTEROCEPTIVE AND INTEROCEPTIVE REFLEXES

COMMUNICATION I. EFFECT OF UNCONDITIONED EXTEROCEPTIVE AND INTEROCEPTIVE STIMULI ON A SPINAL MOTOR REFLEX

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It is known that under normal conditions exteroceptive stimulation is of prime importance in motor system activity. Numerous researches [2, 10, 22, and others] have elucidated the effect of exteroceptive stimuli, such as light, color, sounds of various tones and harmonies, smells, and tastes, on voluntary movements of human subjects (ergograms), or on their capacity for performing work requiring precision of movement. It was found that some of these stimuli exerted a stimulatory, and others an inhibitory effect. It is also known that the activity of the motor system of animals and humans is much less affected by changes taking place within the organism, i.e., by interoceptive stimuli.

Many workers have, however, shown that manipulation of internal organs in the course of operations, or their stimulation under experimental conditions, cause changes in the activity of skeletal muscles [13, 15, 16, 21, 23].

It is also known that stimulation of interoceptors leads to changes in the activity of skeletal muscles, such as in coughing, vomiting, swallowing, micturition, and parturition. According to some clinicians, the effect of interoceptive stimulation on voluntary muscular activity may be particularly pronounced in certain morbid conditions, and may, in a number of cases, be the cause of the development of disturbances of the motor system.

M.R. Mogendovich showed in 1941 [12] that stimulation of certain viscera caused changes in the chronaxie of voluntary muscles.

A systematic study of exteroceptive and interoceptive conditioned reflexes of the skeletal musculature has been undertaken by K.M. Bykov and his associates [7].

V.N. Chernigovskii and his associates have made a detailed study of reflexes released by stimulation of interoceptors, and established the existence of two basic types of effects – a "releasing" and a "correcting" effect [8, 9, 17, 18, 19, 20]. I.A. Bulygin [3, 4] has also shown that various types of internal organ stimulation evoke reflex reactions of skeletal muscles.

It may thus be said that the effects of interoceptors on voluntary muscles, under conditions of acute experimentation, have been investigated very thoroughly. Much less work has, however, been done under conditions of chronic experimentation [5, 11, 16, 23].

Still less attention has been paid to the comparative analysis of the correcting effects of exteroceptive and interoceptive stimulation on voluntary muscles. The only reference to this subject that we have been able to

trace is an abstract of a Doctor's thesis by I.D. Boenko [1]. In his animal experiments, this author determined the chronaxie of limb muscles and of the corresponding areas of the cerebral cortex, before and after various interferences with the organism, and found that the chronaxie changes in the same direction in both these systems, and in the same way, irrespective of whether the animal was subjected to stimulation of exteroceptors or interoceptors.

The present paper is devoted to the comparison of the effects of interoceptive and exteroceptive stimulation on a spinal motor reflex which is already in progress.

EXPERIMENTAL METHOD

The experiments were performed on eight dogs. All the animals were provided with a gastric fistula, and were subjected to a Thiry-Vella operation for exteriorization of the intestine. The unconditioned spinal motor reflex (withdrawal or raising of an extremity) was elicited by electrical stimulation of the sole of a fore or hind limb. We used ac current from the mains, supplied to a rheostat, connected potentiometrically.

The current was switched on for periods of 0.5 seconds, at a frequency of 12 impulses per second. At the beginning of each experiment we determined the threshold by moving the slide of the rheostat, and then slightly increased the current to give a pronounced reaction. The threshold varied from 0.5 to 1.5 v for different animals. We repeated the stimulus 15-20 times, over 5-10 minutes. Respiratory movements, and those of the stimulated limb, were recorded on a kymograph. In addition, electrocardiograms were recorded in some of the experiments.

We were not interested in the electrocardiograms as such, except as a means of registering heart rate.

Interoceptive or exteroceptive stimulation was applied during the period of electrical stimulation of the leg, i.e., while the unconditioned motor reflex was in progress. We called these "extra stimulations." Leg movements were recorded in some experiments by means of a V.P. Petropavlovskii splint, and in others by the method of N.N. Poliakova [14], using a rubber tube partly filled with mercury and connected with a rubber bellows fitted with a writing stylus. We applied the following exteroceptive stimuli: light (L), ringing of a bell (b), intermittent ringing of a bell (int. b), a metronome (M_{100} and M_{300}), a skin stimulator applied to the right shoulder (St.rt.), or to the left hip (St.l.), electric shocks applied to the skin of an ear, or a flank, or of a leg (El.e., El.f., and El.l.), irrigation of the mouth with 0.3% hydrochloric acid (HCl_m). Interoceptor stimulation was effected by dilating the ampulla of the rectum by means of a rubber balloon (R), applying a pressure of 70-80 mm Hg, distension of the stomach (Sto.), by inflating a rubber bag at a pressure of 40 mm Hg, and irrigation of the small intestine with 0.2-0.3% hydrochloric acid (HCl_i). In most of the experiments the duration of action of the stimuli was 25 seconds. In one series of experiments, we applied rhythmic stimulation to the left hind leg, and in another to the left fore leg.

EXPERIMENTAL RESULTS

It is evident from the kymograms that extra stimulation, whether of exteroceptors or of interoceptors, may either not affect the spinal motor reflex (Figure 1: 1, 2, 3, 4), as is found in 15% of the experiments, or it may affect it, as in 85% of the experiments. In those cases in which a correcting effect appeared, the motor reaction which was in progress varied differently for different tests. In some cases, the extra stimulation strengthened the spinal reflex (Figure 1: 5, 6, 7, 8), while in others it weakened it (Figure 1: 9, 10, 11, 12), sometimes completely abolishing it. In other cases, extra stimulation gave rise to a diphasic type of reaction (Figure 1: 13, 14, 15, 16). Restoration of the initial motor reaction followed immediately after discontinuation of the extra stimulation (Fig. 1: 5, 8, 9, 12), or some time after (Figure 1: 10, 11), i.e., after-effects could be observed.

When additional movements appeared (Figure 1: 6, 8), we evaluated the effect as one of stimulation, taking the effect as being one of strengthening of stimulation in the corresponding motor centers. We also considered a halt at the summit of the myogram tracings to be evidence of stimulation (Figure 1: 7). Conversely, we took abolition of spontaneous movements (Figure 1: 10) to be evidence of inhibition.

The respiratory changes were characteristic. The first change was seen on beginning rhythmic stimulation of the limb. If the pulsed current was applied when the animal was breathing regularly, we usually observed an acceleration of the respiratory rhythm, frequently associated with uncoordinated respiratory movements (Figure 1:

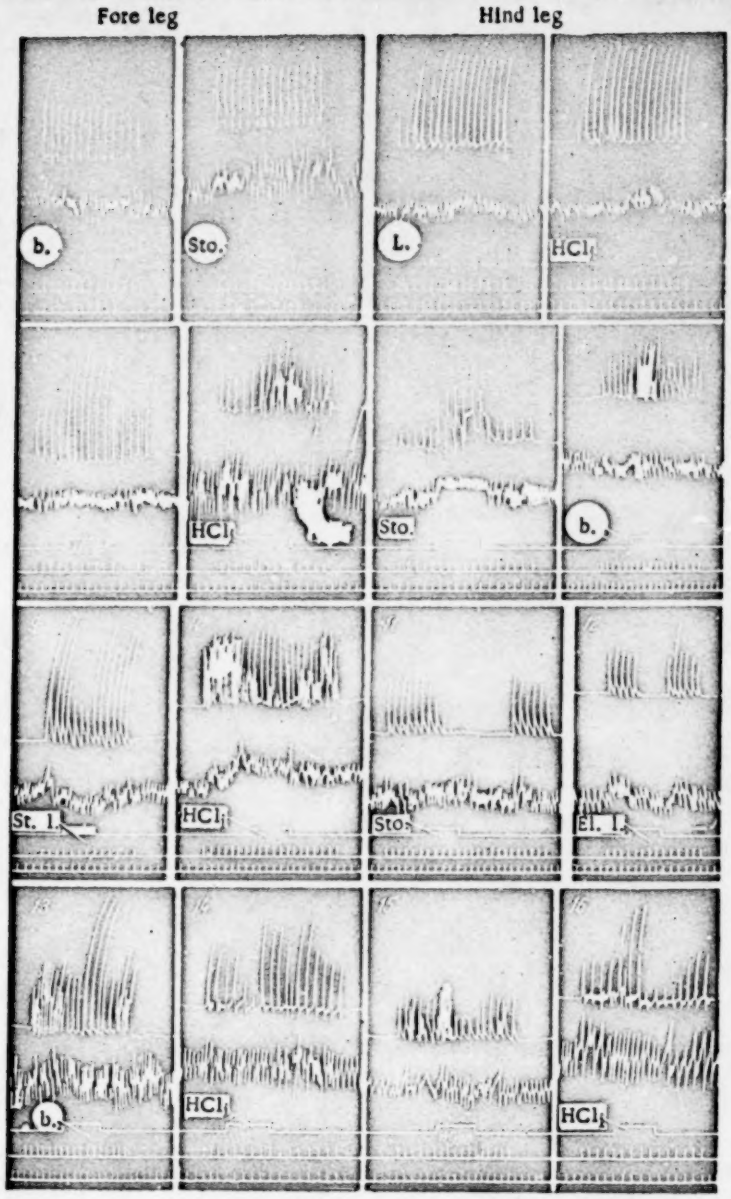

Fig. 1. Effect of exteroceptive and interoceptive stimulation on a spinal motor reflex reaction. Explanation of tracings (from above down): reflex movements of the leg, respiration, extra stimulation signal, rhythmic stimulation signals, time marker (five seconds). Fragments of kymograms 1-16, recording results of tests of different stimuli. Explanation in text.

6, 7, 10, 11). We often observed steep respiratory waves, which appeared at the moment when stimulation was started, and were in synchrony with the electric pulses (Figure 1: 7, 11, 14, 16). When the rhythmic stimulation was applied during a phase of hyperpnea the effect, in the majority of cases, was to abolish the hyperpnea, which was immediately replaced by a much slower respiratory rhythm, which was not in all cases regular (Figure 2: a).

Extra stimulation also affected respiration. The uncoordinating effect of rhythmic stimulation usually disappeared, and in about 60% of the cases a type of "normalization" of respiration ensued for the duration of action of the extra stimulation.

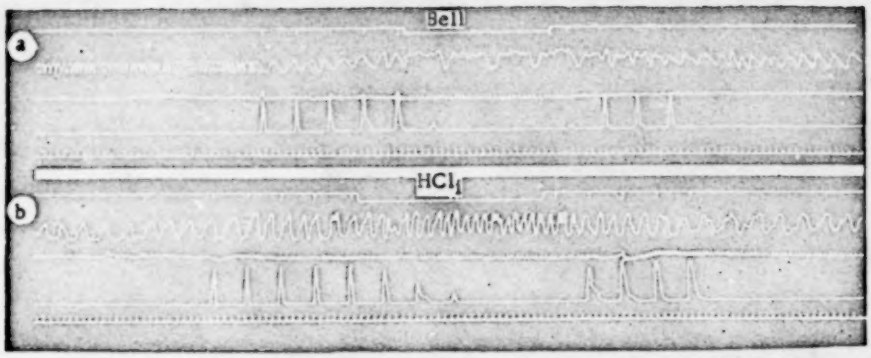

Fig. 2. Effect of rhythmic and extra stimulation on respiration and heart rate. Explanation of tracings (from above down): signals of rhythmic and extra stimulation, respiration, heart beat, reflex movements of the leg, time marker (five seconds).

It is of interest that the respiratory rate again changed during the period of action of the extra stimulus, against a background of rhythmic stimulation. This change consisted, in about half the cases, in a fairly marked retardation of the rate, sometimes with increase in the amplitude of the respiratory movements (see Figure 1: 9, 16). Retardation was particularly pronounced, and most often encountered, with exteroceptive stimulation (Table 1).

TABLE 1

Respiratory Changes During the Action of Extra Stimulation (percentage incidence)

Effect	Interoceptive stimulation	Exteroceptive stimulation	Mean
Retardation	37	63	50
No change	17	13	15
Acceleration	46	24	35

A slight increase in pulse rate appeared when rhythmic stimulation was begun. The superposition of extero- or interoceptive stimulation on a background of rhythmic stimulation usually caused a retardation of heart rate, which in some cases followed a period of acceleration. Acceleration of heart rate again supervened after cessation of the extra stimulus (Figure 2: a, b).

It is evident from the data of Table 2, which shows the percentage incidence in five dogs of inhibitory, stimulatory, and diphasic effects of extra stimulation, that, both for the fore and the hind legs, the most frequently encountered corrective effect of this stimulation on an existing spinal motor reaction is to inhibit the motor reflex, irrespective of whether exteroceptors or interoceptors are stimulated, showing that there is no basic difference between the action of these receptors. This is also shown by Figure 3.

It should, however, be noted that the incidence of inhibition of the motor reflex of the hind leg is higher for interoceptive than for exteroceptive extra stimulation (80%, as compared with 76.4%), whereas the position is reversed for the forelegs (76.5%, as compared with 81%) (see Figure 3: a). We could observe no basic differences between the effects of interoceptive stimulation during rhythmic stimulation of the fore and hind legs. However, here too we found one difference: interoceptive stimulation had less effect on the reflex reaction of the fore than

TABLE 2

Effect of Extra Stimulation On An Existing Spinal Motor Reflex

Exteroceptive stimulation					
hind leg			fore leg		
effect			effect		
inhibitory	reinforcing	diphasic	inhibitory	reinforcing	diphasic
percentage incidence					
82.0	13.8	4.2	82.9	9.5	7.6
80.7	13.2	4.2	82.3	8.0	9.7
71.3	17.7	11.0	78.0	12.4	9.7
69.8	26.6	6.6	–	–	–
78.6	13.8	7.6	–	–	–
76.4	16.4	7.2	81.0	10.0	9.0
Interoceptive stimulation					
hind leg			fore leg		
effect			effect		
inhibitory	reinforcing	diphasic	inhibitory	reinforcing	diphasic
percentage incidence					
72.0	13.0	15.0	78.0	16.5	5.5
83.6	6.4	10.0	77.5	17.5	5.0
87.7	44.9	7.4	73.5	16.5	10.0
77.7	17.7	4.6	–	–	–
79.0	13.0	8.0	–	–	–
80.0	11.0	9.0	76.4	16.8	6.8

of the hind legs. Whereas interoceptive stimulation during rhythmic stimulation of a hind leg had no effect on the spinal motor reflex reaction in 11.6% of the experiments, the corresponding figure for the forelegs was 31.4% (Figure 3: b). The reason for this effect may be that the interoceptive reflex arcs stimulated by us are more closely associated with the hind than with the forelimbs. The greatest incidence of inhibitory effects of exteroceptive stimulation on the motor reflexes of both the fore and the hind legs was found when the stimuli applied were an electric shock to the skin, a skin stimulator, or irrigation of the mouth with hydrochloric acid, i.e., stimuli which had a contact action, while an inhibitory effect was less frequently elicited by stimuli acting at a distance, such as the sound of a bell or metronome, or light. Of the interoceptive stimuli, the most inhibitory was distension of the rectum; the effect of irrigation of the small intestine with hydrochloric acid was smaller, and that of distension of the stomach smaller still.

It appears from the results of our chronic experiments on dogs that extra stimulation emerging from different receptor fields, against a background of an acting spinal motor reflex, exerts a "correcting" action in 85% of the experiments. There is no basic difference between the effects of exteroceptive or interoceptive extra stimulation. In the great majority of experiments an inhibitory effect on the activity of skeletal muscles was seen in both cases.

We cannot say from our experiments whether the vegetative reaction (respiratory and cardiac effects) or the somatic reaction (effect on skeletal muscle activity) appeared first, in response to extra stimulation. Special experiments would be required to provide an answer to this question.

In conclusion we would like to note that, under our experimental conditions, the effects were somewhat more complex than were those reported by authors working under conditions of acute experimentation. Evidently, the higher levels of the central nervous system were involved in the reaction, under our experimental conditions.

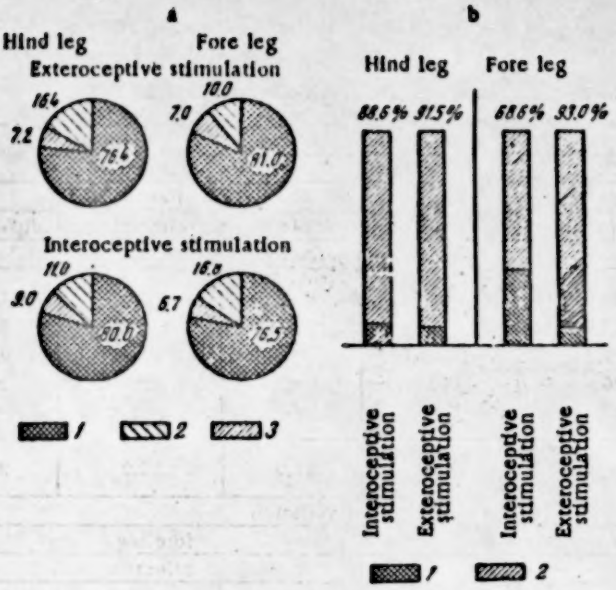

Fig. 3. Effect of extra stimulation on an acting spinal motor reflex: a) effect of exteroceptive and interoceptive stimulation: 1) inhibitory effect; 2) stimulatory effect; 3) diphasic effect; b) presence or absence of effect of extra stimulation: 1) extra stimulation had no effect; 2) extra stimulation had an effect.

SUMMARY

Chronic experiments were performed on dogs. It was demonstrated that stimuli from various fields on the background of a spinal reflex have a "correcting" effect in 85% of the cases. In 80% of cases, this influence is inhibitory, independent of the field from which it was sent. Thus, it may be considered that there is no principal difference in the action of the exteroceptive and interoceptive stimuli on the reflex activity of the skeletal muscles.

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TIME AND CONDITIONS OF PERFORMANCE OF MUSCULAR WORK AS FACTORS OF ORGANIZATION OF DIURNAL PERIODICITY

M.R. Maizelis

From the Leningrad Scientific Research Institute of Physical Culture

(Received April 10, 1957. Presented by Academician K.M. Bykov)

The study of the individual components of daily activity and of their effect in forming the diurnal stereotype of physiological functions, in particular of motor function, is of considerable importance in the physiology of labor and sport.

A large number of papers have been devoted to the study of the diurnal periodicity of motor activity, in relation to nutrition, illumination, temperature, and other factors [1, 2, 3, 4, 6, 8, 9, 12, 14, 15, 16, 18, 19, 20, 21, 22, 23, and many others]. Relatively few papers [5, 7, 10, 11, 13, 17] have dealt with the formation of the diurnal rhythm of motor activity, in connection with muscular activity. But this subject is of particular importance to the study of the physiology of work and of physical training.

In the present paper we have attempted to answer the question of whether the time at which work is performed, and the environmental conditions in which it takes place, are factors affecting formation of diurnal periodicity. We applied the conditioned reflex method to the study of this problem.

EXPERIMENTAL METHOD

Our experiments were performed on long-eared hedgehogs, which under natural conditions display a pronounced diurnal rhythm of physiological function. We examined their motor activities, which were recorded by means of an actograph. The animals were placed in a cage, connected through a corrugated tube with a pressure capsule (tambour). The lever of the tambour was in contact with the surface of a drum, which rotated once in 24 hours. In this way, we recorded any oscillations of the cage due to the movements of the animal.

When under observation, the animals were placed in cages situated in a room in which temperature and illumination were kept constant. Each animal regularly performed a given amount of work (running in a treadmill), at a fixed time of day, and was only removed from its cage for this time. Having become habituated to this routine, the animals needed no inducement to enter the wheel. The mere presence of the wheel acted as a stimulus to active running.

The animals were fed once daily (always at the same time), and were always given the same food. An interval of several hours intervened between feeding and experimental physical work (work was performed in the mornings, feeding in the evenings).

EXPERIMENTAL RESULTS

A. Formation, Within the Diurnal Stereotype, of Conditioned Reflexes to the Time and Place of Performance of Physical Work

Our experiments showed that regular performance of physical work at a fixed time of day caused changes in the diurnal rhythm of motor activity exhibited by the animals at the beginning of the experiment. Thus, we

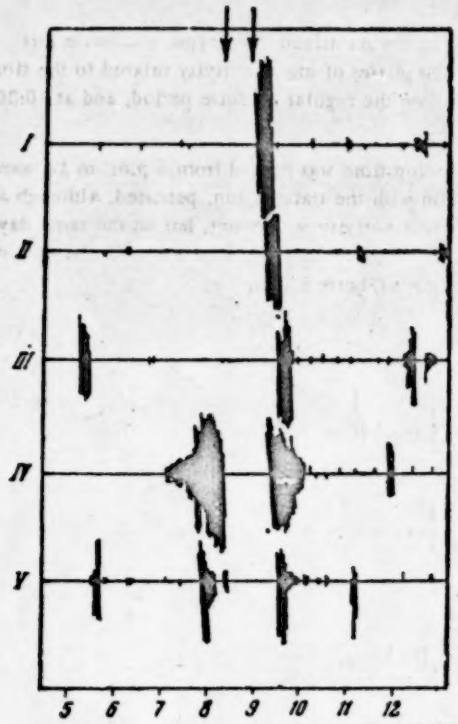

Fig. 1. Formation of a conditioned reflex to the time of performance of physical work (Hedgehog No. 1). Tracings of motor activity. The arrows show the time of placing the animal in the wheel, and of its removal from it: I) experiment of October 26, 1950; II) October 27, 1950; III) October 28, 1950; IV) October 30, 1950; V) October 31, 1950. Below: time of day.

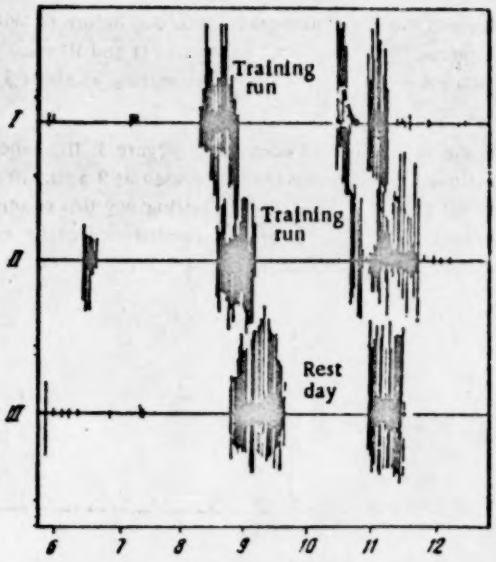

Fig. 2. Conditioned reflex motor activity elaborated in response to physical work (Hedgehog No. 3): I) training day; experiment of October 20, 1950; II) training day; experiment of October 21, 1950; III) rest day; October 22, 1950. Below: time of day.

noticed that after being exercised in the wheel they continued to run backwards and forwards in their cages for a certain time (Figure 1: I, II, III). With continued repetition of their daily training runs, phases of motor activity began to appear in the diurnal cycle, preceding their transfer to the wheel (Figure 1: IV, Figure 2: I, II); these were of the nature of a conditioned reflex to the time of performance of physical work, at a fixed hour of the day.

Once these two phases of activity had been formed within the diurnal stereotype – one preceding, and the other following, the training run – they could be observed not only on days in which such runs took place, but also on days of rest (Figure 2: III, and Figure 3: I, II), which is evidence of stabilization of the experimental diurnal stereotype. Thus, new phases of "spontaneous" motor activity had appeared in the diurnal cycle as a result of regular performance of physical work at a fixed time of day.

It is evident, however, from the recordings, that motor activity was not seen during the period which corresponded with that of the routine training run. But if the animals were taken out of the cages at this time, and raised to the wheel, they jumped in, and began to run. It follows that the motor excitation observed while the animals are in the wheel may be considered to be a conditioned reflex to the environmental conditions. When the animals were left in their cages this reflex was inhibited, owing to the absence of conditions adequate for muscular exertion.

B. Extinction of Conditioned Reflexes to the Time and Place of Performance of Physical Work

A reconstruction of the stabilized diurnal stereotype was effected, with the object of extinguishing the experimentally formed reflexes to the time and place of performance of physical work. For this purpose we discon-

tinued the training runs, and shifted the feeding time to another time of day. All the other experimental conditions remained unchanged.

Figure 3 shows the actograms recorded before reconstruction of the stabilized stereotype, and while this was in progress. The two upper actograms (I and II) show clearly the phases of motor activity related to the time of performance of physical work and appearing at about 9 a.m., before the regular exercise period, and at 10:30-11 a.m., after this period.

On the second day of conversion (Figure 3: III), when the feeding time was moved from 6 p.m. to 12 noon, the conditioned reflex activity usually seen at 9 a.m., in connection with the training run, persisted, although at a slightly different time. On the following day this conditioned reflex activity was absent, but on the same day we observed the appearance of a new conditioned reflex, expressed as motor activity seen between 11 and 12 a.m., i.e., at a time connected with the new feeding schedule of the animals (Figure 3: IV).

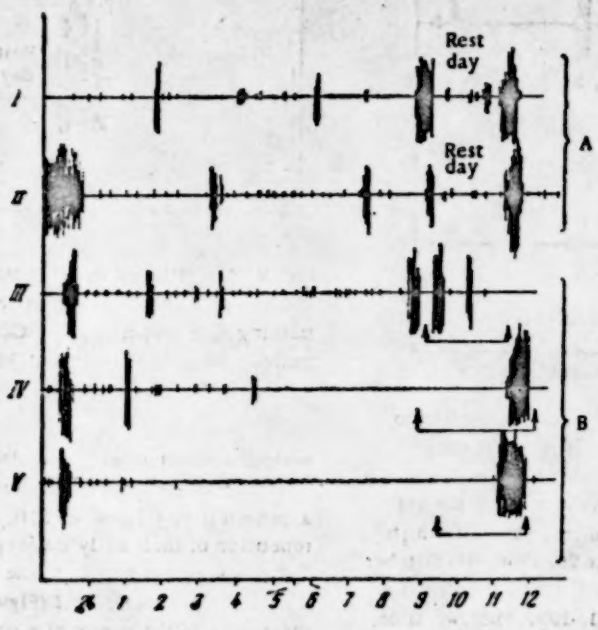

Fig. 3. Extinction of a conditioned reflex to time of performance of physical work, during the process of conversion of a diurnal stereotype (Hedgehog No. 2): A – before conversion of the diurnal stereotype: I) 21st day after beginning of training at a fixed time of day (October 15, 1950); II) 24th day after beginning of training at a fixed time of day (October 18, 1950). B) After conversion of the diurnal stereotype: III) first day of extinguishing the reflex (October 19, 1950); IV) second day of extinguishing the reflex (October 20, 1950); V) third day of extinguishing the reflex (October 21, 1950). Below: time of day.

Discontinuation of the training runs was associated with change in the signal significance of environmental stimuli which coincided with this activity. Thus, during the experiments, signal significance was acquired by sounds connected with the entry into the room of the experimenter (footsteps, opening of the door), and with the noise made by the rotating wheel. The entry of the experimenter into the room, however quietly this was effected, invariably gave rise to increased motor activity of the hedgehogs. It should be noted that the effect of the morning visit of the experimenter was always reinforced by the training run, and of the evening visit by provision of

food. Thus, the experimenter fulfilled the role of a positive stimulating signal, releasing motor activity in the animal. This was very clearly shown on occasions when the animals had contrived to open the cages, from which they escaped, and hid themselves under any available cover in the corners of the room. As soon as the experimenter entered the room, however, they at once abandoned their cover and ran towards him. This behavior was the rule before conversion of the diurnal stereotype. From the moment this was achieved, the signal significance of auditory stimuli connected with the entry of the experimenter underwent change. The evening visit was no longer associated with provision of food. Training runs no longer followed the morning visits of the experimenter.

The motor activity response of the animals to the presence of the experimenter changed accordingly. It is evident from Figure 3 (III) that on the first day of the changed schedule the arrival of the experimenter in the morning, at the time at which the training run used to take place, was associated with the appearance of motor activity. However, on this day the conditioned reflex formed to the time of the training runs was not reinforced by a run in the wheel. On the following day (Figure 3: IV) extinction of the conditioned reflex was seen. The animal evinced no motor activity in response to the entry of the experimenter. The same applied to the next day (Figure 3: V), although the wheel was turned within the room. The cessation of motor activity was found to have taken place some hours before the morning visit of the experimenter.

As feeding time approached (at 12 noon on the new schedule, instead of at 6 p.m. as previously), the animal, which had until then been immobile, got up and moved rapidly around the cage.

Hence, the absence of motor activity, at those times of the day which, before conversion of the stereotype, corresponded with periods of conditioned reflex excitation connected with impending exercise, is evidence of the presence of an active conditioned reflex inhibitory process in the motor analysor.

Thus, the absence of physical exercise which had been performed by the animals at a fixed time (training) converts this time into a negative signal stimulus. In place of excitation, manifested as motor activity of the animals at times corresponding with the former training schedule, conditioned reflex inhibition now supervenes. External stimuli whose incidence coincided with these times now also acquire the significance of negative signals. It is of special interest that the influence of the inhibitory process developing in the diurnal stereotype on extinction of the conditioned reflex to time of performance of physical work was now also apparent during the period preceding that of former excitation. Concentration of inhibition was only achieved on the fourth day of conversion of the diurnal stereotype. Inhibition then applied only to the time of motor activity related, before extinction, to the training run.

Our findings permit the following conclusions.

Stimulation by means of signals of motor activity of an animal is an important factor, determining the organization of any motor activity in time. The time of performance of physical work, and the environment in which it is performed, are components of the process of formation of the diurnal rhythm of motor activity, since they represent signals evoking excitation or inhibition of the motor analysor. The positive conditioned reflexes to time and place of performance of physical work are thus to be included among the causes of apparently "spontaneous" motor activity.

SUMMARY

The investigations established that the time of accomplishment of muscular work and the environmental conditions in which it is performed are the factors which organize the 24-hour periodicity of motor activity. This is due to the fact that both the time factor and the environmental conditions are the signals for the appearance of excitation and inhibition in the motor analysor. Formation of positive conditioned reflexes to time and environment in which the muscular work is performed is one cause of the occurrence of the so-called "spontaneous" motor activity.

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EFFECT OF ANGULAR ACCELERATION ON THE SECRETORY AND MOTOR ACTIVITY OF THE HUMAN STOMACH

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The object of our research was to study the effect of angular acceleration on the secretory activity of the gastric glands of human subjects. We have been unable to find any published work on this subject.

EXPERIMENTAL METHOD

Our experiments were performed on five subjects, aged from 20 to 28, including the author of this paper.

In all, we made 34 observations.

Angular acceleration was achieved by placing the subjects on a centrifuge of radius 3.6 m. The centrifugal force applied varied from 3 to 5 g, acting for 30 seconds in the direction head–seat. The secretory and motor functions of the stomach were observed by means of a double rubber probe, by the method of I.T. Kurtsin. One of the tubes served for collection of gastric juice, and the other for recording motor activity. Stimulation of secretion was achieved by introducing 250 cm³ of air into the rubber bag. The air was released from the bag after an hour, and it was re-inflated after 15-20 minutes, and the secretory and motor activities of the stomach were followed for another hour. Having determined background conditions of gastric secretion and motility, we waited an hour and then gave the subjects a single spin on the centrifuge.

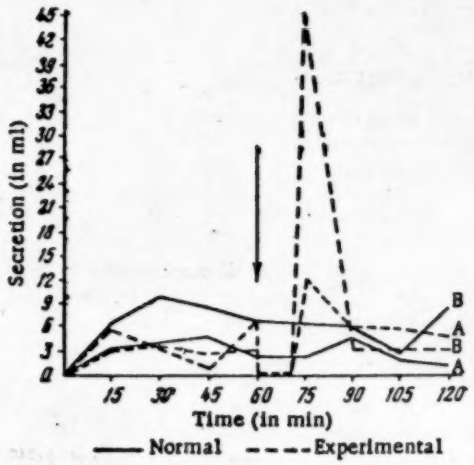

Fig. 1. Effect of a centrifugal force of 3 g, exerted for 30 seconds, on the secretory activity of the gastric glands of two subjects (A and B). Secretion was stimulated by inflating an indwelling rubber bag in the stomach (with 250 cm³ of air). The continuous line applies to secretion under normal conditions, and the broken line to secretion during centrifugation.

With the object of investigating the neuro-chemical phase of gastric secretion, we performed a number of tests following an Ehrman test meal (300 ml of 5% alcohol). The stomach contents were collected after 25 minutes, and secretion was followed during the subsequent hour. Centrifuging was applied immediately after emptying the stomach. We determined the amount of gastric juice, its free and total acidity, and its peptic activity according to Gross.

EXPERIMENTAL RESULTS

We found a considerable difference between the background values obtained after distending the stomach by inflation of a rubber bag, and those observed during the application of centrifugal force.

Figure 1 shows the dynamics of secretion of gastric

juice by two subjects, A and B, subjected to a centrifugal force of 3 g for 30 seconds.

As is evident from the figure, secretion was inhibited during centrifugation, down to total suppression over a period of ten minutes. This was followed by a sharp rise in secretion, to a level 3-5 times greater than the normal values.

Concordant results were obtained for all five subjects when we gave an alcohol test meal; a centrifugal force of 3 g caused inhibition of secretion, followed by an abrupt rise.

In most cases, augmented secretion was accompanied by qualitative changes in the composition of the juice: its free and total acidity and its peptic activity rose. When the angular velocity was raised to 4-5 g, absence of secretion was prolonged to 30 minutes, before giving way to the phase of enhanced secretion. We did not, under these conditions, observe any appreciable changes in the periodic contractions of the stomach (Figure 2). After subjection to a centrifugal force of 5 g (Figure 2: B) they differed little from those encountered initially (Figure 2: A).

Repeated exposure to centrifugation (up to eight times for a single subject, at 5-6 day intervals) led to only very slight smoothing out of the abrupt changes in secretion of gastric juice. Even after the eighth consecutive spin the amount of juice secreted was much higher than the background value. Further work is required before the question of adaptation of the secretory activities of the gastric glands can be elucidated.

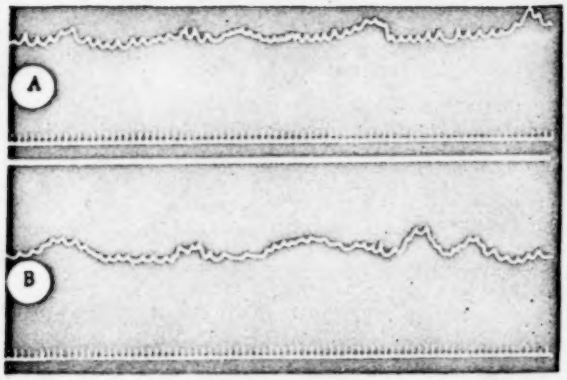

Fig. 2. Periodic contractions of the stomach of an individual (A) before, and (B) after subjection to a centrifugal force of 5 g for a period of 30 seconds. Time marker – three seconds.

It tuus appears that centrifugal forces of the order of 3-5 g, acting in a cranio-caudal direction for 30 seconds, cause considerable alterations in the secretion of the gastric glands. Total suppression of gastric secretion takes place during centrifugation, and persists thereafter for 10-30 minutes, being then followed by a phase of sharp increase (up to 3 times the normal background value) in the volume of juice secreted.

SUMMARY

During the action of radial accelerations and 10-30 minutes after their discontinuation there was complete absence of gastric secretion which was followed by a period of increased secretion (about three times more than in normal conditions). Upon repeated stimulation, only insignificant normalization of these changes took place. The periodic contractions of the stomach remained unchanged.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

SORBENT PROPERTIES OF CERTAIN ORGANS, STUDIED IN EXPERIMENTAL TUBERCULOSIS BY THE SUPRAVITAL STAINING METHOD

COMMUNICATION I. SORBENT PROPERTIES OF THE CEREBRAL CORTEX

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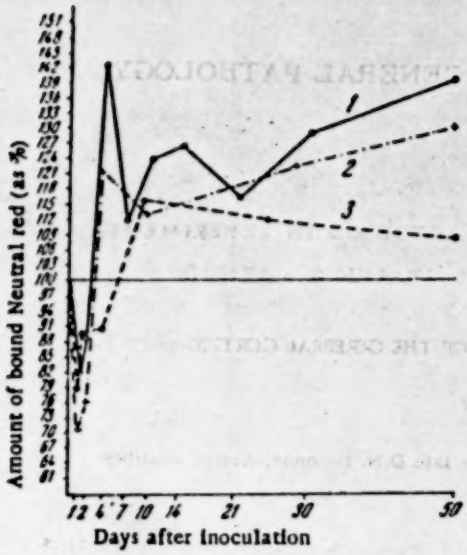
In the study of certain mechanisms of the pathogenesis of tuberculosis, considerable importance is attached to comparative data on the relative resistance of the tissues of different organisms to the pathogen. Although it allows an appraisal of the dynamics of structural lesions of a given organ, the pathomorphological method does not permit the evaluation of early tissue changes, and is still less able to assist in the determination of the precise quantitative extent of injury to the organs at the inception of the morbid process. The necessity arose, in this connection, of finding other methods for the investigation of fine morphofunctional changes in the tissues, arising in the process of development of experimental turberculosis.

It has been shown by D.N. Nasonov and his associates that a variety of injurious factors, differing in their physicochemical nature, give rise in a wide variety of different kinds of cells, to a complex of similar, unspecific changes, to which the term paranecrosis has been applied [7]. One of the most characteristic features of this complex of changes is the more intense staining of the protoplasm of the cells by supravital stains. This unspecific reaction exhibits gradations. The increase in staining capacity of the cells varies directly and quantitatively with the extent of the injury suffered by the cell [1, 2, 4, 5, 6, 8, 9, 10, 11]. It follows that the method of supravital staining of tissues should be applicable to the solution of the problem under investigation. We therefore examined the possibility of applying the method of supravital staining to the evaluation of the extent of injury suffered by the tissue elements of various organs at different stages in the development of experimental tuberculosis. If our results confirmed this possibility, we intended to apply the method to the comparative study of the reactions of the cells of certain organs to tuberculosis infection.

The present paper deals with a study of changes taking place in the sorbent properties of cells of the cerebral cortex of guinea pigs suffering from experimental tuberculosis.

EXPERIMENTAL METHOD

We studied three model forms of experimental tuberculosis, differing in the increasing severity of the pathologic process: disease due to infection with an attenuated BCG culture of M. tuberculosis, disease caused by inoculation of virulent bovine type germs into immunized guinea pigs, and disease due to virulent bovine type germs inoculated into nonimmunized animals.



Curves expressing sorption of dye by tissues of the cerebral cortex of guinea pigs during development of experimental tuberculosis, after inoculation with virulent bovine type bacteria (1), BCG culture (3), and virulent bovine type bacteria after previous immunization (2). The abscissae represent days after inoculation, and the ordinates are amount of Neutral red bound, as percentages. Sorption by brain tissues of control (healthy) animals is taken as 100%.

In the first series of experiments the guinea pigs were inoculated with a suspension of bovine type M. tuberculosis (Strain No. 109) containing 0.01 mg of organisms (in 1 ml of physiological saline), subcutaneously in the region of the right axilla. The animals of the second series were inoculated subcutaneously with 1 mg of BCG organisms (in ml of saline). The guinea pigs of the third series were given two injections of BCG organisms, at an interval of 30 days. Two months after the second injection the animals were given an inoculation of virulent bovine type bacteria (Strain No. 109), at the same dosage level as in the first series.

The animals of the first series were killed 1, 2, 4, 7, 10, 14, 21, 30, and 50 days after inoculation, and in the second and third series 1, 2, 4, 10, 30, and 50 days after inoculation. Healthy guinea pigs, killed at the same times, served as controls. From 5 to 30 animals were taken for examination from the experimental groups, and the same number of controls was taken. We performed 380 examinations in the first series, and 40 each in the other two.

The supravital staining method consisted of the following: the animals were decapitated under ether anesthesia, the cranium was lifted, and the brain was carefully removed and placed first in Ringer's solution for 10-15 minutes, and then into the dye solution (0.01% Neutral red in Ringer solution prepared without sodium carbonate), at 18-20°. The brain was removed after 30 minutes, washed in Ringer's solution, the olfactory lobes and the stump of the spinal cord were removed, and the preparation was immersed for 24 hours in 70° alcohol which had been acidified with 2% sulfuric acid, for extraction of sorbed dye. The brain was then dried to constant weight. The amount of extracted dye was determined by means of a Pulfrich step-photometer, and calculated per mg of dry weight of the brain. The results were subjected to statistical treatment, involving calculation of the mean quadratic error of the deviations.

This procedure permitted the evaluation of the sorbent properties only of the tissue elements of the cerebral cortex, since the dye does not, under the given conditions, penetrate deeper than a few tens of microns below the surface [3]. Microscopic examination of the cortex showed that not only the nerve cells and their processes, but also all the fibers present in this region were stained.

It was necessary, for the evaluation of the sorbent properties of the cerebral cortex of the experimental animals, to know what changes took place in the dry content of this organ during the development of the tuberculous infection. We found, in parallel experiments, that the dry weight of the brain fluctuated only very slightly during the course of the disease. Our findings relating to sorption may therefore be regarded as being trustworthy.

We performed histopathological examinations of internal organs of other guinea pigs parallel with the determinations of sorbent properties of the brain, and at the same times.

EXPERIMENTAL RESULTS

As is evident from our findings (see table and figure), binding of Neutral red by the brains of the experimental animals of all three series very greatly exceeded (by up to 43%) the values found for the control (healthy) animals. This is evidence of a reaction of the tissues of the cerebral cortex, due to the action of a complex of injurious factors (direct toxic and reflex factors), and increasing in intensity as the disease progresses. The greatest intensity of paranecrotic changes in brain cells was found in guinea pigs infected with virulent bovine type

Binding of Dye by Tissues of the Cerebral Cortex of Guinea Pigs Inoculated With Tuberculosis Germs (as percentages of the value found for healthy animals)

Culture taken for inoculation	Time after inoculation (in days)							
	1	2	4	7	10	14	21	30	50
M. tuberculosis	—19.2±4.5	—12.4±3.9	+42.5±6.2	+11.8±3.9	+23.7±5	+26.3±4.6	+16.4±2	+30.2±4.8	+40.6±4.6
BCG	—22±2,8	—11±4.1	—10.3±1.34	— —	+15±5.5	— —	— —	+12±2.3	+8.7±0.66
BCG + M. tuberculosis	—30.3±2.96	—23.7±2.73	+21.3±2.33	— —	+14±5.85	— —	— —	+22.3±3.66	+32±4.61

Note: The symbols + and — represent, respectively, increase or decrease in the amount of dye bound by the cerebral cortex of the experimental animals, as compared with the amount found for healthy control animals. The mean quadratic error is designated by ±. The figures given in each column of the table represent arithmetic means of the results of all the experiments applying to the given time.

bacteria, and the least in those inoculated with BCG culture. Similarly, the most pronounced anatomopathological changes were found in the viscera of animals infected with virulent bacteria; these changes were less pronounced in animals which had been infected with virulent bacteria after immunization with BCG organisms, and were absent from the organs of guinea pigs given BCG vaccinations alone. There was thus a complete parallelism between the severity of the disease and the degree of change in the sorbent properties of the brain.

The change in the sorbent properties of the tissues of the cerebral cortex of the experimental animals was of a biphasic nature. The first phase, seen at the inception of the disease, was characterized by a fall in the capacity of the tissues to bind the dye, while in the second phase there was an increased capacity, shown by the animals of all three experimental series.

The phase in which sorption by the cortical tissues falls below that of the controls, observed within 24-48 hours of inoculation, may, in accordance with the findings of D.N. Nasonov and his associates, be related to adaptation of the brain cells to the action of the complex of factors accompanying the development of tuberculosis. As is evident from the figure, the sorptive capacity of the cortical tissues of animals inoculated with virulent bacteria rose to a value twice as great as in the controls. The first rise in sorptive capacity probably reflects paranecrotic changes which are still partly reversible. In contrast, the second rise in sorptive capacity corresponds with a phase of development of irreversible paranecrotic changes in the brain tissues.

In the experiments involving inoculation with BCG culture, or with virulent bacteria after previous immunization, only one rise in sorptive capacity is seen. This is evidence that the paranecrotic changes occurring in the brain under such conditions differ from those following inoculation with virulent bacteria.

Our findings show that changes in the sorbent properties of the brain originate during the early stages of development of experimental tuberculosis, before any changes in the morphology of the viscera are perceptible (such changes could be perceived seven days after inoculation with virulent cultures, and were not found after BCG inoculation). Our findings show that the supravital staining procedure can serve for the detection of an early reaction of the brain to the development of infection.

We do not consider the changes in supravital staining found by us in guinea pigs inoculated with virulent and BCG cultures necessarily to be specific for tuberculous infection, although the possibility of a certain pathognomicity of these changes for the given pathological process cannot be excluded.

SUMMARY

The method of vital staining was tested in three cases of experimentally induced tuberculosis of varying severity. It was demonstrated that this method was useful in evaluation of the

degree of injury of the tissue elements of the cerebral cortex during the development of this disease. There are no morphological changes which can be detected in the internal organs of animals during the first hours after infection by tuberculosis. However, at that time, substantial changes have already taken place in the cerebral cortex, which are manifested in the change of the sorptive properties of the cells. The degree of change of the sorptive properties corresponds to the severity of the pathological process. The wave-like biphasic character of the changes in the sorptive properties of the cerebral cortical tissue was noted during development of experimentally induced tuberculosis.

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GENERAL AND CARDIOTOXIC ACTION OF O-STREPTOLYSIN

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Two types of streptococcal hemolysins are known, viz., S-streptolysin, which is formed in culture media containing serum, and which does not possess antigenic properties, and O-streptolysin, for the formation of which the presence of serum is not essential.

O-Streptolysin is rapidly fatal to white mice, which die with symptoms resembling those of histamine poisoning [4]. According to some authors [5], no changes in cardiac activity resulted from a single introduction of the product into an isolated frog's heart, but repetition of the dose was followed by cardiac arrest in systole. Other authors [6] found inflammatory changes in the myocardium after parenteral injection of O-streptolysin. Clinical and immunological observations reported by various authors have indicated that in many cases O-streptolysin aggravates the course of streptococcal infection [3].

For all these reasons we thought it would be of interest to make a more detailed study of the toxic properties of O-streptolysin.

EXPERIMENTAL METHOD

Except for experiments involving the use of isolated frogs' hearts, we used concentrated preparations, obtained from filtrates of streptococcal cultures by the method of fractional precipitation with ammonium sulfate (added at first to a saturation of 35% to remove inactive material, and then to 70% to precipitate O-streptolysin). In experiments with isolated frogs' hearts we used preparations made by the method of A.P. Konikov [1]. Titration of O-streptolysin with antistreptolysin was also carried out by the procedure described by this author.

Various doses of O-streptolysin, all in 0.5 ml of solution, were administered by intravenous injection into white mice. Injections of O-streptolysin which had been inactivated by boiling were given simultaneously to control mice.

EXPERIMENTAL RESULTS

All the animals died after receiving doses of 216 units or more, and at least 50% died after receiving 150-180 units. All the animals of the control groups survived the injections. Death was preceded by dyspnea, flow of bloodstained fluid from the nostrils, and rigors. At autopsy, the lungs were found to be edematous, the liver, spleen and kidneys were hyperemic, and blood was frequently found in the urinary bladder. In the great majority of cases death ensued within 10-20 minutes of the injection.

Rabbit antistreptolytic immune serum neutralized the action of O-streptolysin, if given in equivalent amounts. Thus, in one of our experiments we gave 600 units of O-streptolysin, amounting to 3 MLD, together with an amount of antiserum exceeding the neutralizing dose by 10%. All the mice survived injection with this

mixture. This detoxicating action on O-streptolysin was not exhibited by antifibrinolytic or normal rabbit serum.

Some of the sera taken from rabbits immunized with streptococcus vaccine also displayed some neutralizing action.

The specific protective action of antistreptolytic serum was clearly demonstrated in experiments on passive immunization.

To one group of mice we gave injections of 0.5 ml of antistreptolysin, containing 500 units, equivalent to 4500 O-streptolysin units, or 21 MLD. To a second group we gave 0.5 ml of serum from rabbits immunized with streptococcus vaccine. On the following day, the animals of both groups received 3 MLD of O-streptolysin. All the animals of the first group survived, but all those of the second group died.

The kymogram shown in Figure 1 illustrates the action of O-streptolysin on an isolated frog's heart. Introduction into the heart of an O-streptolysin preparation containing 900 units in 1 ml was quickly followed by arrest of the heart in systole. The toxic effect of O-streptolysin was manifested in all the experiments after its first introduction, when given in high dosage. The heartbeat was not restored by washing.

Fig. 1. Effect of a massive dose of O-streptolysin (↑, 900 units) on an isolated frog's heart. Time marker 30 seconds.

Perfusion of the heart with preparations of O-streptolysin containing 20 units in 1 ml was rapidly followed by reduction in the amplitude of the contractions, with subsequent arrest in diastole. Washing out restored the heartbeat.

Repeated perfusion with the same preparation, after washing out the heart three or four times, as was done by Bernheimer and Cantoni [5], did not cause arrest of the heart in systole.

In these experiments neither the first nor the second subthreshold dose of O-streptolysin caused more than a certain retardation of the action of the heart, with its subsequent arrest in diastole. Washing out the heart restored its action (Figure 2).

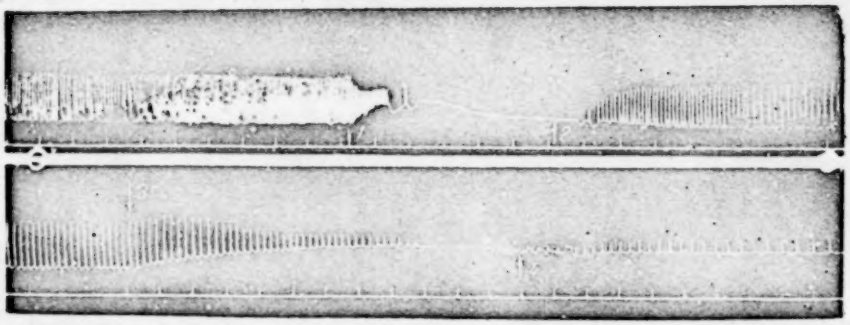

Fig. 2. Effect of repeated small doses of O-streptolysin on an isolated frog's heart: 1,3) introduction of 20 units of preparation; 2, 4) washing out with Ringer solution. Time marker: 30 seconds.

Numerous repetitions of the experiment gave the same result. We were not able to observe any biphasic action of O-streptolysin, or any sensitizing action of the first dose, as reported by the above authors.

No perceptible effect on the heartbeat followed administration of preparations of O-streptolysin inactivated by boiling.

We have not been able to trace any references in the literature to the action of O-streptolysin on the isolated heart of mammals. Yet the results of such investigations could be of more value in the study of the pathogenesis of some streptococcal infections than are those obtained using the heart of cold-blooded animals. The kymogram of Figure 3 illustrates the effect of introducing 7000 units of O-streptolysin into the perfusion stream; a period of acceleration of short duration was followed by spastic contraction of the myocardium, and by arrest in systole. Relaxation of the heart muscle could not be achieved by prolonged perfusion with the nutrient medium.

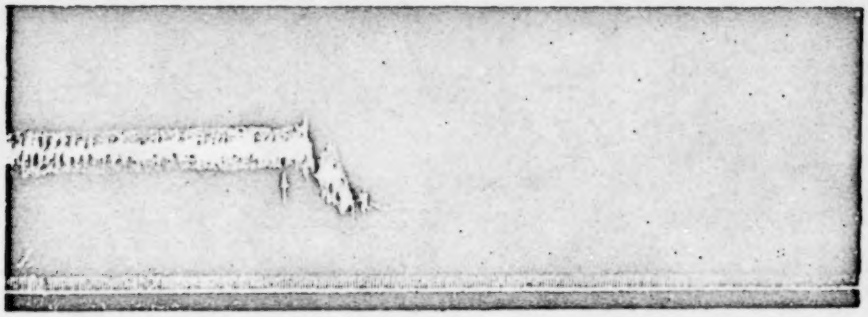

Fig. 3. Effect of O-streptolysin (7000 units) on an isolated cat's heart. Time marker: one second.

These effects were not seen when O-streptolysin inactivated by boiling was introduced into the perfusion fluid. The boiled preparation caused a considerable, though transient, increase in the amplitude of the heartbeat, after which it continued to contract normally for a long time, although more feebly than before boiled O-streptolysin was given. The kymogram of Figure 4 was recorded during one of these experiments. We repeated the introduction of boiled O-streptolysin (1500 units) three times. After each introduction of boiled preparation an increase in amplitude of the heartbeat was seen, without subsequent arrest of the heart.

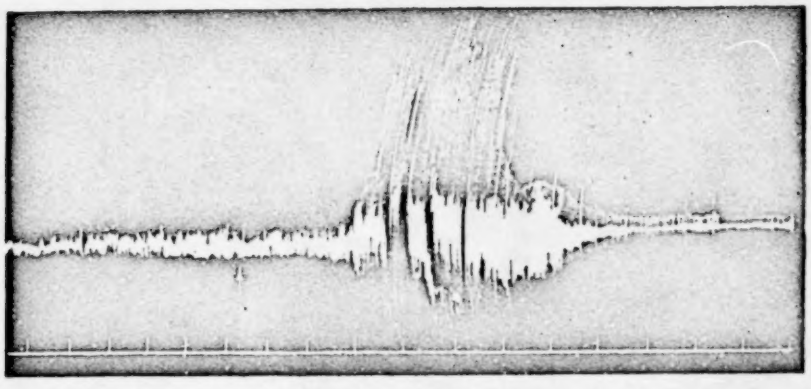

Fig. 4. Effect on an isolated cat's heart of 25 mg of boiled O-streptolysin ↑ Time marker: 30 seconds.

The specificity of action of O-streptolysin was also shown in experiments with erythrogenic toxin and with fibrinolysin. Introduction of large doses of these preparations (15,000 units and 2 ml, respectively) into the perfusion fluid did not cause any appreciable changes in the activity of the isolated heart.

In the next series of experiments we examined the effect of repeated small (subthreshold) doses of O-streptolysin. The first dose caused strengthening of the heartbeat, of short duration; it was followed by a gradual restoration of the normal rhythm. The same effects followed the second dose. The third dose caused arrest of the heart in systole.

The toxic action of O-streptolysin on the isolated cat's heart was abolished by previous addition to it of an equivalent amount of antistreptolytic serum. Introduction of such mixtures into the perfusion fluid affected the rhythm and the strength of the cardiac contractions, but did not lead to arrest of the heart.

Sera obtained from rabbits immunized against other streptococcal antigens (erythrogenic toxin, fibrinolysin, streptococcal vaccine) had no similar effect on the toxicity of O-streptolysin, with the exception of one batch of antifibrinolytic serum.

We examined the effect of O-streptolysin on the isolated heart of animals which had previously been immunized against this toxin. The results were not clear-cut, probably because of the differences in the degree of immunity of different individuals. In some of the experiments the effect of O-streptolysin in no way differed from that found for the heart of nonimmunized animals. In other experiments, the action of O-streptolysin was weaker; the myocardial spasm normally supervening after arrest of the heart did not appear.

Our study of the toxic properties of preparations of O-streptolysin on animals confirm published reports of its general toxic action on white mice. However, published figures for the minimum lethal dose for mice vary within very broad limits – from 10 units [4] to 4000 units [5]. Our value for the minimum lethal dose falls between these extremes.

These differences may be due to differences in sensitivity of mice of different strains to the toxic action of O-streptolysin. It may, however, be that the differences between the values for the minimum lethal dose reported by the various authors depend on the degree of purification of the preparations used by them, since they may contain different amounts of other streptococcal products, the introduction of which, into the organism, is not necessarily without effect. At the same time, there can be no doubt that O-streptolysin exerts a specific toxic action, not dependent on the possible presence of other streptococcal products. This is shown by the observation that strictly equivalent amounts of antistreptolytic serum neutralized its toxic action.

Arrest of an isolated cat's heart after repeated administration of O-streptolysin seems to have been the result of its cumulative action, since the total amount introduced in these experiments equalled the dose required for manifestation of the typical effects of this toxin. The specificity of the cardiotoxic action of O-streptolysin was established in experiments with other streptococcal toxins, and by experiments involving its neutralization by antistreptolytic immune serum and by other antistreptococcal immune sera.

SUMMARY

O-streptolysin has a general toxic effect and causes quick death when injected intravenously in white mice.

The cardiotoxic effect of O-streptolysin was studied in experiments on isolated hearts of frogs and cats. It was demonstrated that O-streptolysin brings about cardiac arrest and contraction of the heart muscle. However, streptococcal antigens do not give such an effect on the isolated heart. The toxic action of O-streptolysin may be neutralized by the specific immune serum. The influence of O-streptolysin on the isolated heart of immune animals differed from its action on the hearts of normal animals in a number of experiments.

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PATHOGENESIS OF DYSENTERY

COMMUNICATION I. EFFECT OF DYSENTERIC INTOXICATION ON THE ASCORBIC ACID METABOLISM OF WHITE RATS

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A serious hindrance to the study of the pathogenesis of dysentery has been the absence of any satisfactory experimental model of dysenteric infection. Introduction of cultures of dysentery germs into laboratory animals does not give rise to the disease in them, and excretion of the bacteria ceases after a few days. It is known that the reason why small laboratory animals cannot be infected with dysentery is related to the intricate complex of mechanisms of natural immunity, not all of which have yet been discovered.

We have, over a number of years [2], been engaged in the study of the distribution of ascorbic acid (AA) in the organs of animals exposed to the action of external and internal factors which affected the reactivity of the organism, and we invariably found that the AA content of the organs deviated from the normal values, and that each separate factor caused a specific change in the pattern of distribution of AA in the organs. We found no change in the ascorbic acid content of the organs when the given factor did not release any of the defense mechanisms of the organism.

In the present paper we describe the results of an examination of the effects on the AA content of various organs, due to administration of dysentery cultures or toxin to white rats, maintained on an adequate and a deficient diet. Changes in the distribution of AA in the organs of the rats were taken as indicators of changes in their reactivity towards the dysenteric infection, and of the effect of dietary factors on the susceptibility of the animals to the given pathogenic agents.

EXPERIMENTAL METHOD

We used white rats for our experiments. The animals were divided into six groups. A dose of 40 billion organisms, taken from a 24-hour agar culture of Flexner dysentery bacteria, was administered through a stomach tube to the rats of the first group (eleven rats), which received the ordinary laboratory stock diet. The rats were killed by decapitation three days later. The second group (six rats), also receiving the stock diet, were given a subcutaneous injection of 2 ml of Boivin's complete antigen prepared from the dysentery bacteria. The animals of the third group (ten rats) were given dysentery culture by stomach tube as well as antigen subcutaneously, at the above dosages. This group was on the same diet as the preceding ones. The fourth group (fifteen rats) served as controls for the first three groups, and received the same diet, but neither culture nor toxin was administered.

The animals of the fifth group (twelve rats) were maintained on a low-protein diet until they had lost 20% of their initial weight, when dysentery culture was given, at the above dosage level; the rats were killed three days later.

Changes in the AA Content (mg %) of Various Organs of Rats, Following Administration of Dysentery Culture or Toxin

Group	Liver		Small intestine		Large intestine		Spleen		Cerebral hemispheres		Kidneys		Suprarenal glands	
	M±m	T	M±m	T	M±m	T	M±m	T	M±m	T	M±m	T	M±m	T
First (stock diet) Given dysentery culture	26.0±1.0	0.8	34.9±2.0	1.7	30.9±1.4	1.5	40.6±2.7	1.0	44.4±1.2	4.3	17.8±1.1	1.1	486.3±26.5	4.0
Second (stock diet) Given toxin	25.5±0.5	0.6	41.2±0.9	4.4	36.9±1.9	3.7	44.6±1.5	3.4	43.8±0.5	5.2	18.2	(n, inadequate)	426.8±	(n-inadequate)
Third (stock diet) Given culture + toxin	30.3±1.5	2.7	42.3±1.0	5.0	33.4±0.9	3.1	48.3±1.1	5.8	45.1±1.5	4.2	20.7±1.6	2.8	456.6±18.7	5.7
Fourth (stock diet) Controls	24.4±1.6	—	29.5±2.5	—	27.2±1.9	—	37.1±1.6	—	37.1±1.2	—	16.4±0.4	—	332.3±27.3	—
Fifth (low-protein diet) Given dysentery culture	17.8±1.1	1.5	32.2±2.3	1.8	26.0±1.6	3.5	38.4±3.1	3.0	40.5±1.2	4.5	14.1±1.3	4.5	386.0±27.6	3.2
Sixth (low-protein diet) Controls	14.6±1.9	—	24.6±3.5	—	17.0±1.9	—	23.7±3.9	—	32.4±1.2	—	7.1±0.8	—	256.3±29.8	—

The sixth group (eleven rats) served as a control for the fifth group; the rats were maintained for the same length of time on the same low-protein diet, but were not given dysentery culture. These rats were killed at the same time as were those of the fifth group. The low-protein diet consisted of casein 3%, corn starch 78%, sunflower seed oil 9%, Osborne-Mendel salt mixture 4%, dry baker's yeast 5%, and fish oil 1%.

The following organs were taken for analysis for AA immediately after decapitation, using our previously described method [1]: liver, small and large intestine, spleen, the cerebral hemispheres, the kidneys, and the suprarenal glands.

EXPERIMENTAL RESULTS

It is evident from the results tabulated in the table that most of the organs of rats receiving the stock diet, viz., the liver, small and large intestine, spleen, and kidneys, had the same AA content after administration of dysentery culture as were found in the control group. Significant rises in AA content were found in the brain and in the suprarenal glands.

A much more pronounced reaction was seen after injection of toxin into the rats. In this group, we found significant increases in AA content of the wall of the small intestine ($T = 4.4$) and of the large intestine ($T = 3.7$), of the spleen ($T = 3.4$), and of the cerebral hemispheres ($T = 5.2$). The number of determinations of AA in the kidneys and adrenals was insufficient for statistical treatment. The AA content of the liver remained at normal levels.

A more intense reaction followed simultaneous administration of dysentery culture and toxin. This was manifested by a considerable rise in the AA content of all the organs taken for analysis.

The conclusion may be drawn from our results that the organism of rats is not unreactive either to dysentery bacteria or to dysentery toxin.

Under conditions of protein deficiency the rat organism was found to be far more sensitive to introduction of dysentery culture than was that of rats maintained on the stock laboratory diet. As is evident from the table, administration of dysentery culture was followed by a considerable and significant rise in the AA content of the walls of the large intestine, of the spleen, of the cerebral hemispheres, and of the kidneys, very considerably exceeding the rises in AA content of the same organs after administering dysentery culture, or even toxin, to animals previously maintained on the stock diet.

The results of this experiment provide evidence that dietary factors are of considerable importance in determining the reactivity of small laboratory animals to dysenteric intoxication.

The conclusion may be drawn from the changes in AA content of the various organs that the central nervous system and the adrenal-sympathetic system are the first to be involved in the pathologic process under conditions of mild intensity of attack (administration of dysentery culture to animals on the stock diet). Under more acute conditions (administration of toxin to rats receiving stock diet, or administration of dysentery culture to rats maintained on a protein-deficient diet) the blood cells (as shown by the changes in AA content of the spleen) and the intestines are also affected. The least affected organ is the liver.

In view of our findings regarding the significance of dietary factors in the pathogenesis of dysentery, work has been instituted on this aspect of the problem in monkeys.

SUMMARY

Dynamics of the concentration of ascorbic acid in the organs upon introduction of Flexner dysentery culture, and the total bovine antigen recovered from dysentery bacilli was used as an indicator of the change of reactivity of the organism. In rats receiving a full diet (i.e., animals which are considered nonsensitive to dysentery infection) this test reflects the effect of the central nervous and adrenal-sympathetic systems. Injection of the toxin causes more pronounced reaction with involvement of the blood system and intestines. The effect is much more pronounced when the culture is introduced into rats which were kept for a long time on a diet with low protein content. Thus, the condition of nutrition plays an important part in the pathogenesis of dysentery.

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THE EFFECT OF HEPARIN ON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS

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The reports in the literature on the effect of heparin on the serum cholesterin and phospholipid contents are contradictory. Herzstein (1954) et al. [11], and Raynaud and his co-workers [16] consider that heparin does not affect the level of cholesterin and phospholipids in the blood, whereas other workers [7, 4] have found that heparin raises the serum cholesterin level of patients with xanthomatosis and atherosclerosis.

Graham et al. [10] showed that heparin causes conversion of higher classes of lipoproteins with a flotation rate Sf 10-30 into lower (Sf 5-10) and so on, as a result of which, in the author's opinion, there is inhibition of the development of experimental atherosclerosis in rabbits fed on cholesterin.

No less contradictory are the experimental results [9, 12, 13, 14, 15] on the effect of heparin on the development of atherosclerosis, although the majority of authors conclude that heparin inhibits the development of atherosclerosis in experimental animals.

It is possible that the difference in the experimental results of the various workers is due to the use of different doses of heparin. For this reason we set out to investigate the effect of large and small doses of heparin on the level of the blood cholesterin and phospholipids and on the development of atherosclerosis in rabbits during prolonged feeding with cholesterin.

EXPERIMENTAL METHOD

Experiments were performed on 53 chinchilla rabbits weighing 2100-2600 g. All the rabbits were kept under identical conditions and on the same diet. Atherosclerosis was produced by N.N. Anichkov's method of feeding the animals for 100 days with a solution of cholesterin in sunflower oil (at the rate of 0.2 g of cholesterin per 1 kg body weight).

Heparin was injected intravenously every day in different doses into the experimental animals (28 rabbits): 15 rabbits received heparin in a dose of 30 mg, seven rabbits received 60 mg and six rabbits 3 mg. The controls (23 rabbits) received cholesterin only.

In order to ascertain the effect of heparin on the content of endogenous cholesterin and lecithin, two rabbits were injected every day with 30 mg of heparin intravenously.

Every 25 days the serum cholesterin content was estimated by the Engel'gardt-Smirnova method [6] and the phospholipids by the sulfite-hydroquinone method [2] with extraction by Bloor's method [8].

When 100 days had elapsed the animals were killed by air embolus of the lungs. At postmortem examination the aorta and heart were extracted. The aorta was fixed in 10% formalin solution and then stained with Sudan III in toto.

The degree of the atherosclerotic changes in the aorta was designated by + signs (0 – no changes; + – slight changes; ++ – moderate changes; +++ – severe changes, and ++++ – very severe changes).

A study of the effect of a single injection of heparin on the blood lipids of rabbits was carried out at various periods of cholesterin feeding. From 5-6 ml of blood was taken from the marginal vein of the rabbit's ear before and 30 minutes, 1-2 and 3 hours after the intravenous injection of heparin.

EXPERIMENTAL RESULTS

After a single injection of heparin, in not one of the 22 cases was any increase in the cholesterin level observed.

In four cases there was a reduction in the cholesterin cnntent by about 10%, which we regard as within the normal limits of variation of the cholesterin level. In 18 cases, the reduction in the cholesterin varied between 12.5 and 32% of the original value.

The fall in the blood cholesterin content was observed 30 minutes after the injection of heparin and lasted for three hours. In some cases, after an initial fall in the cholesterin level it was observed to rise at the end of the second hour of the experiment, to fall again at the end of the third hour. The effect of a single injection of heparin on the level of the blood cholesterin is shown in the form of curves in Figure 1.

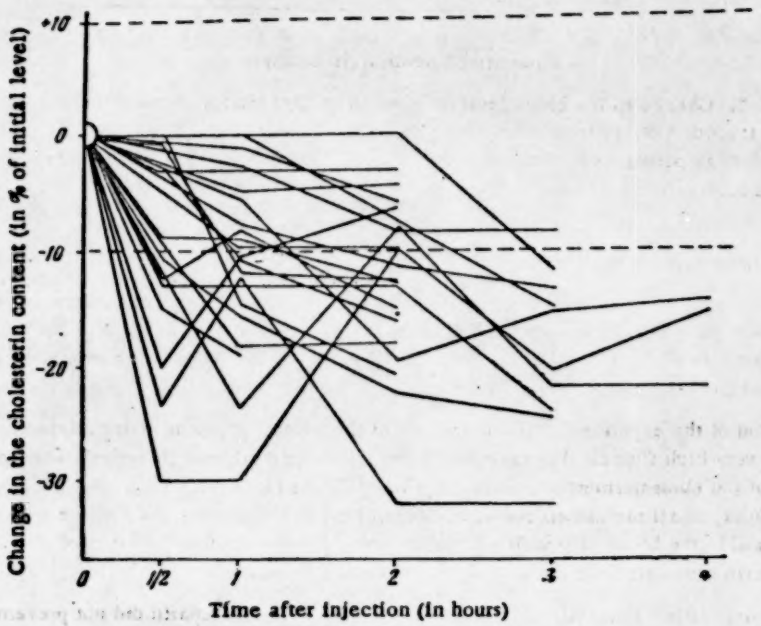

Fig. 1. Change in the blood cholesterin of rabbits after a single intravenous injection of heparin. The curves represent the changes in the cholesterin content in percentages of the initial level; the dotted lines show the normal limits of variation of the blood cholesterin.

Mention must be made of the relation between the degree of the reduction in the cholesterinemia and its initial level: the higher the initial blood cholesterin level the greater the degree of its fall. The magnitude of the fall in the blood cholesterin level did not depend on the dose of heparin used.

The lecithin content fell after injection of heparin (Figure 2) to approximately the same extent as the cholesterin content, so that the value of the coefficient lecithin/cholesterin was practically unchanged.

Thus, a single injection of heparin into rabbits with alimentary hypercholesterinemia led to a reduction in

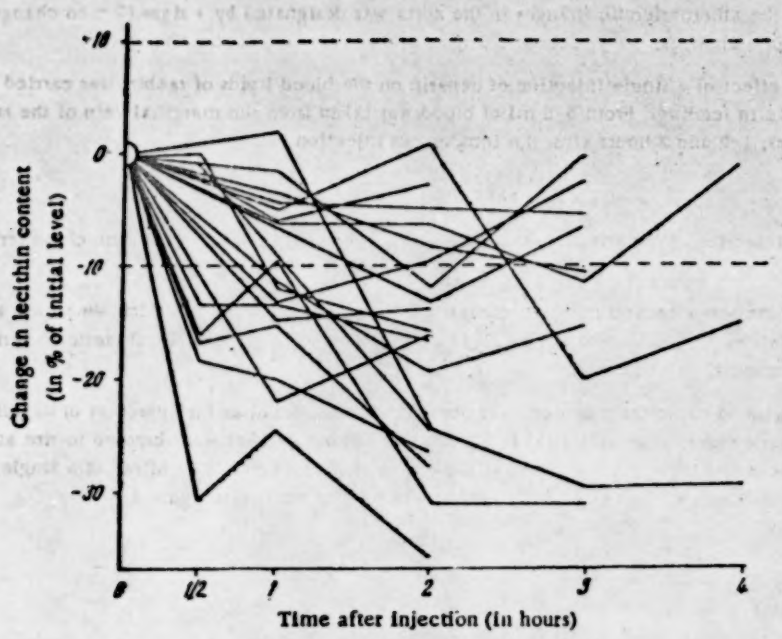

Fig. 2. Change in the blood lecithin level of rabbits after a single intravenous injection of heparin. The curves represent the changes in the lecithin content in percentages of their initial value; the dotted lines show the normal limits of variation of the lecithin level.

the level of cholesterin and lecithin in the blood without any essential change in the lecithin/cholesterin ratio.

Prolonged administration of heparin was well tolerated by the animals. No difference was observed in the weight of the rabbits of the control group receiving cholesterin alone, and the animals of the experimental group receiving cholesterin and heparin. One rabbit only, receiving heparin in a dose of 60 mg, died on the 27th day of the experiment from gastrointestinal hemorrhage.

Towards the end of the experiment all the animals of the control group developed hypercholesterinemia, sometimes reaching very high figures. In rabbit No. 3, for instance, the blood cholesterin level rose to 2560 mg%. The average value of the cholesterinemia in this group was 1071.5±113.3 (Figure 3). Besides the development of hypercholesterinemia, in all the rabbits the blood lecithin content also rose. Its average value was 630.7±65.7. The degree of increase in the blood phospholipid content was considerably less than that of cholesterin, so that the lecithin/cholesterin ratio fell from 2.3 to 0.59±0.028 (average values).

Prolonged administration to rabbits of both large and small doses of heparin did not prevent the development of hypercholesterinemia. In the group of rabbits receiving small doses of cholesterin and heparin the average level of the blood cholesterin was higher (1295±209) than in the control group (Figure 3). The average values of the blood lecithin content were 690±95.7. The value of the lecithin/cholesterin coefficient in these rabbits was the same as in the animals of the control group.

In rabbits receiving large doses of heparin (60 and 30 mg), the average level of the blood cholesterin at the end of the experiment was 980.7±51.1 and 749±36 (see Figure 3), i.e., somewhat lower than in the control group, but the difference was not significant. Injection of large doses of heparin did not affect the rise in the blood lecithin content. The average lecithin level was 688±75.1 and 474±49.6. The mean values of the lecithin/cholesterin ratio in these groups of rabbits were slightly higher than in the control group (0.7±0.09 and 0.75±0.06), but this difference also was not significant.

The administration to rabbits of heparin alone in a dose of 30 mg intravenously had no effect on the level of endogenous cholesterin and lecithin.

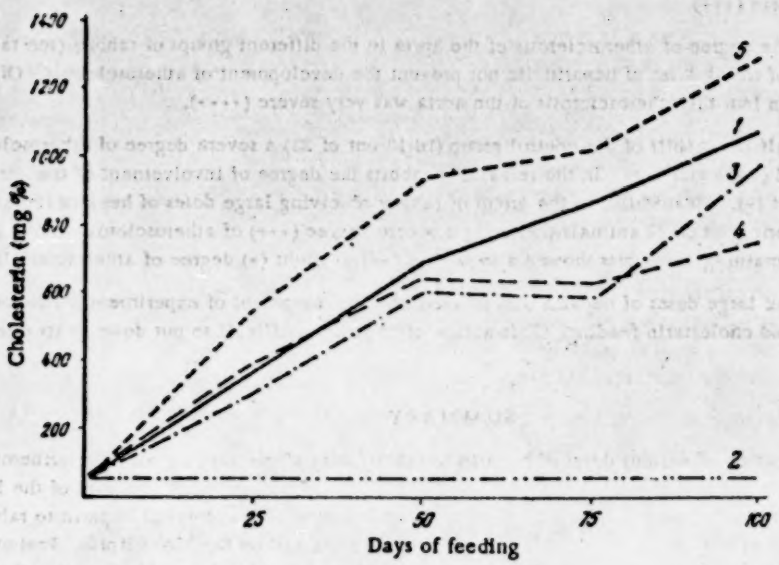

Fig. 3. Development of hypercholesterinemia in various groups of rabbits after prolonged feeding with cholesterin: 1) cholesterin – control; 2) heparin – control; 3) cholesterin + heparin 60 mg; 4) cholesterin + heparin 30 mg; 5) cholesterin + heparin 3 mg.

It should be mentioned that in the control group and in the group of rabbits receiving cholesterin and heparin in a dose of 30 mg, the blood cholesterin content of three animals either did not rise at all (rabbit No. 9) or rose only slightly in the last month of feeding (rabbits Nos. 4 and 52).

There are reports of such "cholesterin resistant" rabbits as these in the articles by S.M. Leites [3], and T.A. Sinitsyna and T.N. Loviagina [5].

Atherosclerotic changes in the aorta were shown in varying degree in all the experiments, except in rabbit No. 9 in which, as mentioned above, no increase in the blood cholesterin level was observed in spite of prolonged feeding with cholesterin.

Large atherosclerotic patches were situated mainly in the region of the arch of the aorta and smaller ones around the orifices of the intercostal arteries. In cases of well-marked atherosclerosis lipoid deposits were observed throughout the whole extent of the aorta.

The Degree of Atherosclerosis of the Aorta in the Various Groups of Rabbits

Agent applied	Number of rabbits in the group	Degree of atherosclerosis of the aorta				
		++++	+++	++	+	0
Cholesterin (control)	23	2	11	5	5	–
Cholesterin, heparin						
60 mg	7	–	1	3	3	–
30 mg	15	–	2	5	7	1
3 mg	6	4	1	1	–	–

On comparing the degree of atherosclerosis of the aorta with the magnitude of the hypercholesterinemia a parallel trend can be observed between them in the majority of cases. However, in some cases, the degree of atherosclerosis of the aorta did not correspond to the height of the hypercholesterinemia. In rabbit No. 82 for instance, with a blood cholesterin level of 1600 mg % the degree of lipoidosis of the aorta was insignificant (+),

while in rabbits Nos. 33 and 34, with hypercholesterinemia of 500 and 580 mg % the degree of atheromatosis of the aorta was very severe (+++).

On comparing the degree of atherosclerosis of the aorta in the different groups of rabbits (see table) it is clear that the giving of small doses of heparin did not prevent the development of atherosclerosis. Of the six rabbits in this group, in four the atherosclerosis of the aorta was very severe (++++).

In more than half the rabbits of the control group (in 13 out of 23) a severe degree of atherosclerosis of the aorta was observed (++++ and +++). In the remaining rabbits the degree of involvement of the aorta was moderate (++) or slight (+). Meanwhile, in the group of rabbits receiving large doses of heparin (60 and 30 mg) in addition to cholesterin, out of 22 animals there was a severe degree (+++) of atherosclerosis of the aorta in only three, and the remaining 19 rabbits showed a moderate (++) or slight (+) degree of atherosclerosis.

Administration of large doses of heparin thus inhibited the development of experimental atherosclerosis in rabbits during prolonged cholesterin feeding. This action of heparin is difficult to put down to its effect on the blood lipids.

SUMMARY

Single administration of various doses of heparin to rabbits with alimentary hypercholesterinemia brought about the decrease in the cholesterol and lecithin blood level to the same degree. The value of the lecithin/cholesterin coefficient remained almost unchanged. Prolonged introduction of low doses of heparin to rabbits fed with cholesterin had no effect on the degree of atheromatosis of aorta and on the blood lipids. Prolonged intravenous introduction of large doses of heparin (30 and 60 mg) inhibited the development of aortic atherosclerosis in rabbits but had no definite effect on the content of the blood serum lipids.

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PHTHIVAZID AND CHOLESTERIN METABOLISM

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The biosynthesis of cholesterin in the body is effected by a number of reactions as a result of which are formed high aliphatic hydrocarbons (squalene), which later undergo cyclic transformation into a phenanthrene ring [3]. The chain of this precursor of cholesterin is formed as a result of the action of acetyl-coenzyme A [7].

Study of the effect on metabolism of the derivatives of isonicotinic acid hydrazide, to which group phthivazid belongs, has shown that these drugs may interfere with the formation of acetyl-coenzyme A [1, 4].

TABLE 1

Serum Cholesterin Content in Patients with Pulmonary Tuberculosis Treated with Phthivazid

Serial No.	Patient's name	Cholesterin (mg %)	
		before giving phthivazid	on the 3rd day of phthivazid therapy
1	B-a	155	134
2	P-o	162	162
3	R-ia	130	120
4	B-n	166	144
5	G-s	125	90
6	Kh-o	148	126
7	K-k	157	119
8	Sh-ch	144	125
9	Zh-v	165	165
10	P-a	174	133
11	Ia-k	133	124
12	K-i	161	130
13	P-a	147	106
14	S-o	168	126
15	Ia-o	100	89
16	B-o	139	96
17	K-sh	120	99
18	Sh-a	134	111
19	L-o	158	144
20	S-ch	134	119
21	M-v	160	148
22	S-v	94	85
23	L-a	114	132
24	L-v	148	131
	Average	143	123

There is evidence of possible weakening of the resynthesis of ATP during isoniazid therapy [9]. The most likely explanation of this is that withdrawal of the acetyl residue for the requirements of acetylation of the isoniazid [5, 6] may affect the synthesis of cholesterin.

In the literature accessible to us we were unable to find any investigations of this problem, yet changes in the serum cholesterin content are often used by phthisiologists as a reliable indication of the control of the course of a tuberculous infection: it is generally considered that with successful treatment of tuberculosis the serum cholesterin content rises.

EXPERIMENTAL METHOD

The aim of our investigation was to study the changes in the serum cholesterin content in a group of patients with pulmonary tuberculosis (of the chronic fibrocavernous type) under treatment with phthivazid. Estimation of cholesterin was done on admission of the patients to hospital before starting phthivazid and later (in the majority of patients) on the third day of therapy (1 to 1.5 g per day). A small group of patients was examined on the 3rd, 15th and 30th days of treatment.

At the beginning of the investigation all the patients were in a phase of flare-up of infiltration. We did not separate the patients according to the form and extent of the lesion, since the action of phthivazid on cholesterin metabolism was the same in all the patients.

EXPERIMENTAL RESULTS

As a rule on the third day of phthivazid therapy the serum cholesterin level fell. Out of 24 patients examined the drug showed this effect in 21. In two patients the serum cholesterin level remained unchanged and only in one did it rise (Table 1).

During observations for one month (eight patients) it was found that the cholesterin level gradually begins to rise, but even on the 30th day of treatment, on the day before discharge, when the majority of the patients were in a satisfactory condition, the serum cholesterin concentration did not exceed its original level (Table 2).

TABLE 2

Serum Cholesterin Content in Patients with Pulmonary Tuberculosis Treated with Phthivazid

Serial No.	Patient's name	Before giving phthivazid	Day of phthivazid therapy		
			3rd	15th	30th
1	K-i	161	130	147	154
2	S-o	168	126	139	159
3	B-o	139	96	119	124
4	K-sh	120	99	–	121
5	S-ch	134	119	136	122
6	M-v	160	148	141	164
7	S-v	94	85	92	109
8	L-v	148	131	166	–
	Average	140	115	134	136

The results obtained are confirmed by others reported in the literature. A case has been reported of the use of isoniazid in treating cholesterin pleurisy arising in a patient with tuberculosis. The course of treatment led to an alteration in the character of the disease, with a fall in the cholesterin concentration [8]. It is likely that phthivazid acts in the same way as isoniazid. High doses of phthivazid also lower the cholesterin level [2].

Thus, changes in the serum cholesterin level in patients with pulmonary tuberculosis are the result not only of the pathological process but also of drug therapy, and this must be taken into account by the clinician.

SUMMARY

The authors studied the effect of phthivazid on the synthesis of cholesterin in the organism of tuberculous patients. It was established that in the majority of patients (21 of the 24) the therapeutic doses of phthivazid decrease the cholesterin level from 143 mg % (before administration of phthivazid) to 123 mg %, on the average by the third day. With prolonged treatment by phthivazid (one month) the cholesterin level gradually approaches its initial value (but never exceeds it).

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THE DISTURBANCE OF VASCULAR PERMEABILITY IN BURNS. AN ELECTROPHORETIC INVESTIGATION OF THE PROTEIN CONTENT OF LYMPH FLOWING FROM THE BURNED AREA

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In previous work [2] we showed that in dogs with localized thermal burns of the skin (1-3% of the body surface) of the first and second degree, sustained 24 hours previously, albumin labelled with radioactive iodine (I^{131}) and injected intravenously appears more quickly in the lymph of the thoracic duct, and the concentration of labelled albumin rises in the tissues outside the burned area. On the basis of these facts, it was concluded that in thermal burns of the skin, besides local disturbance of the permeability of the capillaries in the burn itself, the permeability of the capillaries in tissues and organs remote from the burn also increases, which we [3, 4] and I.A. Mukhamedzhanov [5] observed in experimental ammonia abscesses of the skin.

In order to elucidate the pathogenesis of the general changes in capillary permeability which we observed in burns we studied the protein content of lymph flowing from the burned area and compared it with that of the blood serum.

EXPERIMENTAL METHOD

The investigation was carried out on 37 rabbits of different color and sex weighing from 1.2 to 1.8 kg.

The burn was produced by immersion of the hind paw of the rabbit for one minute in water at a temperature of 80°C.

The lymph for the electrophoretic investigation was obtained from the popliteal lymphatic gland in which a cannula was inserted against the lymph flow.

The protein composition of the lymph serum was investigated with the aid of electrophoresis on filter paper with a potential gradient of 17-18 v/cm, a veronal buffer at pH = 8.6, $\mu = 0.023$. The strips of paper were stained with a 0.5% solution of bromphenol blue prepared in a saturated alcoholic solution of mercuric chloride. The dye was extracted with a 0.01 N solution of caustic soda and the eluted dye examined photometrically by means of a graduated photometer with an M-57 filter. Curves were drawn in accordance with the optical densities. In the calculations and correction of the albumin tracing we followed the instructions of E.P. Smolichev [9]. The composition of the serum proteins of the blood was studied by the same method.

The protein concentration in the lymph was determined by an IRF-1 immersion refractometer.

EXPERIMENTAL RESULTS

The results of the determinations of the protein composition of the lymph from the popliteal gland of healthy rabbits and of rabbits with burns of varying duration are shown in Table 1.

TABLE 1

Content of Protein and Protein Fractions in the Lymph Flowing from the Burned Limb (mean results of experiments on 37 rabbits)

Statistical index	Protein (g%)	Albumin	Globulins					A/G coefficient
			α_1	α_2	β_1	β_2	γ	
Normal lymph (mean values from 10 experiments)								
M	2.1	61.9	9.8	7.3	8.1	—	12.9	1.7
σ±	0.7	6.0	4.8	4.4	5.1	—	3.8	0.6
m±	0.22	1.90	1.23	1.07	1.60	—	1.20	0.15
60 minutes after burning (mean values from experiments on seven rabbits)								
M	3.2	61.3	7.7	5.6	7.5	7.7	11.2	1.6
σ±	0.34	2.4	3.6	2.7	2.6	2 8	1.6	0.2
m±	0.13	0.9	1.4	1.0	1.0	1.1	0.6	0.07
90 minutes after burning (mean values from experiments on seven rabbits)								
M	3.8	59.9	7.9	5.7	7.2	6.1	12.3	1.5
σ±	0.5	4.9	3.0	2.3	1.7	2.1	2.1	0.2
m±	0.2	1.8	1.2	0.9	0.7	0.6	0.6	0.08
24 hours after burning (mean values from experiments on thirteen rabbits)								
M	4.3	49.0	15.1	8.4	8.2	7.0	12.4	1.0
σ±	0.6	5.3	2.5	3.4	2.2	3.4	2.9	0.2
m±	0.2	1.4	0.7	0.9	0.7	1.0	0.8	0.05

The flow of lymph in early stages after the burn had been inflicted was slow, so that the lymph was collected for about 60 minutes and it was impossible to study its composition immediately after the burn. Twenty-four hours after the burn, the paw was very hyperemic and edematous. The skin fold on this paw was from seven to eight times thicker than that on the normal paw. In all cases the lymph flow was accelerated so that it was possible to collect the quantity required for electrophoresis in five to ten minutes.

The protein concentration in the lymph was increased (P less than 0.01) in the first one to two hours after the burn. The lymph collected during the first hour after the burn was found to contain an additional β-globulin fraction (β_2-globulin). The relative content of this fraction did not vary during 24 hours. The relative content of albumin and other globulin fractions was essentially unchanged in the lymph during the first one to two hours after the burn. After 24 hours the protein concentration in the lymph rose sharply (P less than 0.01), the relative albumin content fell (P less than 0.01) and the α_1-globulins rose (P less than 0.01). The A/G ratio was reduced correspondingly (P less than 0.01).

The results of the investigation of the protein composition of the blood serum are shown in Table 2.

Sixty minutes after burning, a tendency of the β-globulin to increase was observed (P less than 0.1). It is possible that this was due to the appearance of the additional β_2-globulin fraction which could not be isolated because of its low concentration in the blood.

DISCUSSION OF RESULTS

In 1955, T.S. Paskhina showed that the ability of the exudates to disturb capillary permeability does not show itself because of the inhibitory effect of albumin [6, 7, 8]. Other authors [12, 14, 15] came to the same conclusion. M.S. Surovikina [10] discovered in the blood of rabbits during experimental inflammation the appearance of an additional β_2-globulin fraction, and this was confirmed by T.S. Paskhina [7, 8]. M.S. Surovikina began her investigation of the protein composition of the blood serum 24 hours after the application of ammonia. Accordingly, D.E. Al'pern [1] considers that active proteins are characteristic of inflammation but are not responsible for it.

TABLE 2

Content of Protein and Protein Fractions in Blood from the Auricular Vein During a Burn (mean results of experiments on seven rabbits)

Statistical index	Protein, g %	Albumin	Globulins				A/G coefficient
			α_1	α_2	β	γ	
Before burning							
M	6.4	61.9	7.5	7.3	9.2	14.0	1.6
$\sigma\pm$	1.5	4.2	2.1	4.8	3.7	3.6	0.3
$m\pm$	0.6	1.6	0.8	1.8	1.4	1.4	0.1
After 5 minutes							
M	6.2	61.1	7.4	6.6	10.8	13.8	1.6
$\sigma\pm$	0.33	4.6	2.1	2.0	2.8	4.4	0.3
$m\pm$	0.12	1.7	0.8	0.7	1.0	1.7	0.1
After 20 minutes							
M	6.1	60.0	8.4	7.0	10.4	11.8	1.5
$\sigma\pm$	0.39	3.8	3.5	1.5	5.1	3.7	0.8
$m\pm$	0.15	1.4	1.3	0.6	2.0	1.4	0.3
After 40 minutes							
M	6.1	59.8	8.5	6.9	10.2	14.5	1.5
$\sigma\pm$	0.33	4.7	2.8	1.6	2.5	4.4	0.8
$m\pm$	0.1	1.8	1.0	0.4	0.9	1.7	0.3
After 60 minutes							
M	5.9	58.6	7.8	7.8	13.2	12.4	1.5
$\sigma\pm$	0.38	6.1	1.6	2.9	5.5	3.4	0.4
$m\pm$	0.14	2.3	0.6	1.1	2.1	1.3	0.1

Our experiments demonstrate the appearance of additional β_2-globulins in the lymph in the initial phase of development of the burn. This is a convincing argument against the view of D.E. Al'pern, and one which confirms the conclusions of T.S. Paskhina and other authors, pointing out the importance of the active β-globulins as substances capable of causing the development of disturbance of capillary permeability in a focus of inflammation. Evidently, these substances are formed in the inflammatory focus and, on entering the blood, gradually lead to the appearance of the β_2-globulins discovered in rabbits. It may be supposed that the disturbance of capillary permeability outside the inflammatory focus is caused by circulation of these active β-globulins in the blood. Increase in the capillary permeability outside the inflammatory focus can be found in rabbits in the first days of development of an ammonia abscess [5]. It is possible that the action of the active globulins is gradually inhibited on the appearance in the body of substances possessing anti-inflammatory activity [13].

In putting forward this view of the role of the β_2-globulins in the production of general disturbance of capillary permeability in inflammation we do not deny that other substances, for example proteinases, whose content in the blood is increased during inflammation, may also play a part in this process [11].

SUMMARY

Electrophoretic investigations of the lymph draining from burned rabbits' paws were performed. It was established that the concentration of protein increases within the first hour after the burn and an additional β-globulin fraction (β_2-globulin) appears. In 24 hours, the derangement of the protein content of the lymph is even more pronounced, i.e., the concentration of protein increases, the relative content of albumin diminishes, β_2-globulin fraction is still present and the level of α_1-globulin is also raised. In accordance with literature data on the effect of active β-globulins on the local capillary permeability the author suggests that these proteins play

an important role in the appearance of general disturbances of capillary permeability in the areas free from inflammations. These disturbances were revealed by the author in experimentally induced inflammation in rabbits and dogs. They were described in his previous papers.

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THE EFFECT OF DISTURBANCE OF EXTERNAL RESPIRATION ON RENAL FUNCTION

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An important aspect of the problem of the pathology of external respiration is the connection between dyspnea arising in the upper part of the respiratory tract and disturbance of the function of various organs and systems of the body.

The explanation of actual forms of disturbance of the activity of the body in cases of embarrassment of external respiration is very important from both the theoretical and practical points of view. The latter is concerned with the necessity of ascertaining the character and mechanism of the disturbances of certain functions of the body in cases of stenosis of the trachea and larynx resulting from various acute and chronic diseases. However, in pathological physiology the study of the effect of difficulty in the passage of air through the trachea and larynx on various functions of the body is mainly concerned with embarrassment of external respiration of such a degree that it is accompanied by the manifestations of asphyxia; of all the various functions of the body the only ones which have been subjected to any detailed study are respiration and the circulation of the blood. Nevertheless, it is a fact that other functions may be disturbed during embarrassment of respiration, and the character and degree of these disturbances must be determined by the intensity and duration of the dyspnea on the one hand and by the characteristic features of the reaction of the respective organs and tissues to the dyspnea on the other.

It must be thought that the appearance of a disturbance in the activity of various organs and systems of the body during embarrassment of external respiration (stenoses of the trachea and larynx) must be determined in the first place by the anoxia which arises in certain forms of stenosis. In addition, it must be borne in mind that difficulty in the passage of air through the trachea or larynx is accompanied by disturbance of the character of the air circulation in various parts of the upper respiratory tract – the nasal cavities, the accessory sinuses and the larynx, i.e., where disturbance of the air circulation is usually connected with stimulation of nerve receptors and reflex changes in the most widely differing regions of the body as a result [2, 3, 4, 9, 13].

On the question of in what cases do what mutual relationships between these factors determine the development of disturbance in the activity of a particular organ or system, we have evidence from actual examples of the development of stenosis and of some peculiarity of regulation of a certain organ or system of the body. Investigations carried out in this direction in the laboratory of pathological physiology of the State Research Institute for the Ear, Nose and Throat have revealed a number of relationships governing the effect of acute and chronic stenosis of the trachea and larynx on the function of the circulatory and digestive systems and have shown the importance of anoxia and of disturbance of the pattern of respiration in the upper respiratory tract [12, 14].

We set ourselves the task of studying the character and mechanism of the effect of various forms of disturbance of respiration in chronic tracheal and laryngeal stenosis on the excretion of urine by the kidneys.

That this is a reasonable problem for investigation is suggested by the fact that the excretory function of

the kidneys is affected on the one hand by lack of oxygen in the inspired air [1, 7, 8, 15], and on the other hand by disturbance of the character of the air circulation in the upper respiratory tract [5, 6, 9, 11].

EXPERIMENTAL METHOD

Experiments were performed on dogs with exteriorization of the ureters separately under the skin by the I.P. Pavlov-L.A. Orbeli method. The present paper deals with experiments on nine dogs.

The excretion of urine by the kidneys was investigated in these animals while on a high intake of water and milk. The renal function was assessed by the volume of urine collected every 5 or 20 minutes (depending on the experimental conditions) for a period of four hours. Separate determinations were made of the glomerular filtration and the tubular reabsorption from the creatinine clearance. For this purpose the endogenous creatinine of the blood and the urinary creatinine of the animals were determined before the production of stenosis in the trachea, during stenosis and at various times after its removal. Calculation of the glomerular filtration and of the tubular reabsorption was made by Rehberg's formula. In all the experiments the content of protein and blood in the urine was determined.

The obstruction to the passage of air through the trachea and larynx was created in the experiments of long duration by introducing a tracheotomy tube into the trachea through a preliminary tracheotomy. Closure of the tracheotomy tube allowed the degree of obstruction to respiration to be regulated. Thus, in these experiments nose breathing was impossible. In experiments in which nose breathing was preserved stenosis was produced by tightening up a special clamp made of stainless steel previously applied to the exposed trachea. With the aid of this clamp it was possible to produce any desired degree of stenosis of the trachea as quickly and for as long as necessary.

Stenosis of the larynx was produced by injection of liquid paraffin under the mucous membrane, thereby diminishing the lumen of the larynx. In all the experiments a kymographic tracing of the respiration was made by means of a cuff applied to the thorax. The respiration was recorded before the application of the stenosing device, during stenosis and after its removal. The chronic experiment required the reapplication of stenosis to the same animal, and so we systematically checked the renal function as revealed by the above-mentioned indices in these animals during normal respiration before the reapplication of the stenosing device.

EXPERIMENTAL RESULTS

The study of the effect of transient stenosis of the trachea on the excretion of urine by the kidneys showed that the character of the changes in renal function depend on the severity of stenosis. We subdivided the stenosis according to its duration into four groups. The first group included stenosis lasting from one to ten minutes, the second from ten minutes to one hour, the third from one to five hours and the fourth from one to three days. According to its severity the stenosis was classified in three categories – severe, moderately severe and slight. The severity of stenosis was determined by the character of the tracing of respiration on the drum of the kymograph and by the clinical picture of stenosis which was sufficiently characteristic of each category.

The experiments showed that in stenosis lasting up to ten minutes and belonging to the severe category, the disturbance of excretion of urine by the kidneys was characterized by a severe fall in diuresis, and in some cases by its complete cessation, which took place either at the same time as or a few minutes after the onset of stenosis. In these cases a compensatory increase in excretion of urine appeared with complete regularity, starting a short time after removal of the stenosis; thus, the total urinary excretion throughout the whole period of the experiment with stenosis of this duration and severity did not differ substantially from the urinary output under normal conditions (Figure 1). With this severity and duration of stenosis, profound disturbances of urinary excretion appeared in some animals in the form of the appearance of protein in the urine, and in some cases of blood. If the stenosis of this duration belonged not to the severe but to the moderately severe category, the changes in renal function as a rule were rather less marked, but the fall in diuresis was always clearly marked, while the compensatory increase in diuresis arising a short time after removal of the stenosis was less pronounced but constant (Figure 2).

In cases of stenosis of the same duration but of slight severity (dyspnea appeared in the animals only on physical exertion), usually no essential changes appeared in the renal function.

In experiments set up to study the effect of renal function in which stenosis lasted from ten minutes to one

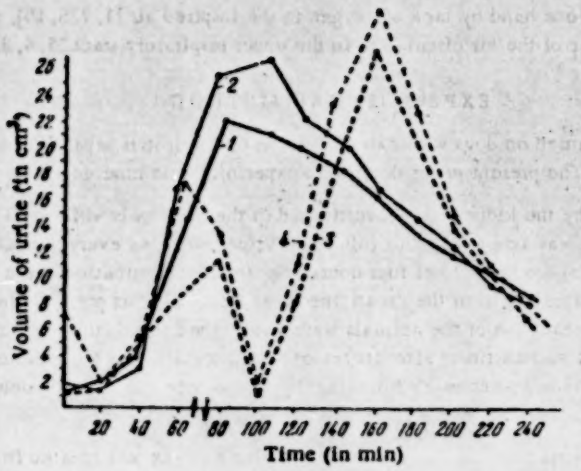

Fig. 1. Diuresis in the dog Mamasha in normal conditions and during severe acute stenosis of the trachea lasting five minutes (high water intake): 1) normal diuresis of the left kidney; 2) normal diuresis of the right kidney; 3) diuresis of the left kidney during the stenosis experiment; 4) diuresis of the right kidney during the stenosis experiment; ↑ – beginning of stenosis; ↓ – end of stenosis.

hour, we found that in its general features the change in the excretory function of the kidneys was the same as in the case of the stenosis of shorter duration – up to ten minutes.

The results of the effect of stenosis lasting several hours on the urinary excretion are particularly interesting. Whereas stenosis lasting for several minutes or tens of minutes (in the two previous groups) was produced during a period of increased excretion of urine following the high fluid intake or else was brought to an end immediately after the diuresis began to fall, stenosis lasting more than one hour occupied a much greater proportion and in some cases the whole of the time of the experiment, i.e., covered the time both of the rise and fall

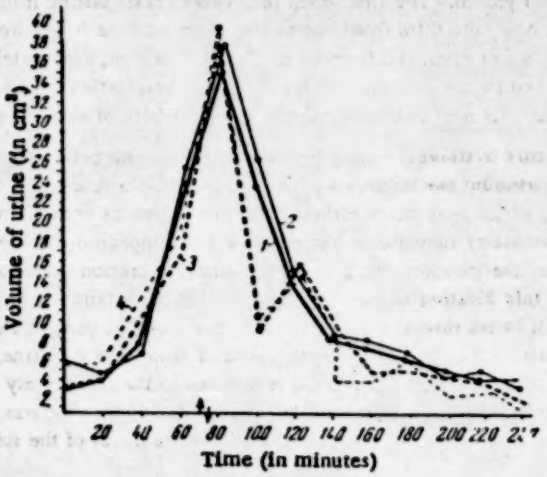

Fig. 2. Diuresis in the dog Chernushka in normal conditions and during stenosis of the trachea of moderate severity lasting five minutes (high water intake). Conventional signs as in Figure 1.

in the output of urine. Under these conditions it was found (and this is most important) that, in spite of the fact that stenosis applied before the intake of water continued for the whole period of the experiment (four hours or more), the diuresis followed the same curve as in cases where stenosis lasting a few minutes ended at the moment of maximum increase of excretion of urine following intake of water: at first there was a sharp fall in diuresis and immediately afterwards a compensatory rise, although the obstruction to respiration (severe stenosis) still continued. Figure 3 shows the curves of urinary output from which it is clear that although stenosis was applied be-

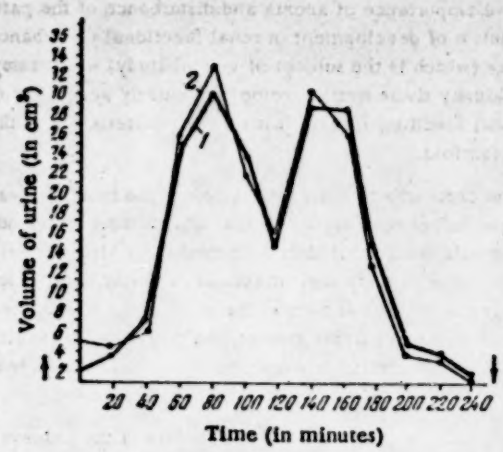

Fig. 3. Diuresis in the dog Mamasha during stenosis of the trachea produced before a high intake of water and lasting throughout the whole experiment. Conventional signs as in Figure 1.

fore the water intake, both kidneys reacted to this water intake normally by an increase in diuresis reaching a maximum at the beginning of the second hour, after which the diuresis diminished considerably, as was observed in stenosis lasting a few minutes. Later the diuresis rose, giving a compensatory reaction similar to that observed in the previous series of experiments after removal of the stenosis. In this case, this compensatory reaction of the kidneys appeared during continuing severe stenosis of the trachea. A similar picture was observed in experiments in which the stenosis produced was of the same duration but of moderate severity.

As in the series of experiments in which the stenosis was of short duration, in the more prolonged stenosis the fall in diuresis and its subsequent compensation were less pronounced in moderately severe stenosis than in severe, but they were always clearly present. When stenosis lasting one hour did not start before the water intake but after it, its effect on diuresis was conditioned by yet another factor: if the onset of stenosis preceded maximum diuresis or coincided with it the diuresis usually underwent further modification in the form of suppression and subsequent compensatory increase, so that the total output of urine remained within normal limits during the experiment; if this type of stenosis began after the diuresis had reached its maximum and had started to fall, no clearly marked suppression of urine excretion nor subsequent compensatory reaction ensued.

It is natural that absence of this type of diuretic reaction should be observed not only in case of slight or moderate stenosis but also in severe stenosis.

It is thus quite clear that the character of the reaction of the excretory function of the kidney to transient stenosis depends on the period of renal activity during which the stenosis arises: if it develops during the period of maximum renal activity during the period of greatest elimination of water taken into the body, then it is accompanied by clear and regular changes in the urine-excreting function of the kidneys in the form of suppression of diuresis and subsequent compensatory increase. These changes are not observed however, in cases where stenosis of this sort arises during diminished renal activity in the period after the maximum excretion by the kidneys of water taken into the body.

What explanation of this fact can be given? The finding that in general the kidneys react only to tran-

sient, severe stenosis or to moderately severe stenosis and do not react to slight degrees of stenosis suggests that kidney function is adequately resistant to embarrassment of external respiration, or in any case is more resistant than the secretory and motor function of the stomach and especially the function of the heart, as can be seen by comparing our findings with those of A.I. Iunina and I.B. Kholmatov from our laboratory. The resistance of the kidneys to obstruction of the passage of air through the trachea is related, obviously directly, to the resistance of the kidney tissues to oxygen lack.

Leaving for a while the relative importance of anoxia and disturbance of the pattern of respiration in the upper respiratory tract in the mechanism of development of renal functional disturbances during obstruction of the passage of air through the trachea (which is the subject of special study) we can nevertheless consider that the manifestations of anoxia in the kidney tissue must develop particularly acutely in cases where stenosis coincides with the time of maximum renal function, i.e., with maximum diuresis, for at this time the oxygen demand of the kidney tissue increases fourfold.

It can be understood from these facts why stenosis arising before the intake of water is not reflected at all in the first phase of excretion of urine before maximum diuresis, and on the other hand produces characteristic changes in diuresis always after a definite interval of time – immediately after diuresis has reached its maximum. It must be supposed that before the kidneys develop their maximum activity, i.e., before maximum diuresis, the kidney tissue can cope with the oxygen lack; but as soon as the work of the kidneys reaches a maximum (maximum diuresis) and the oxygen demand is several times greater, the compensatory ability of the kidney tissue is exhausted and reactions appear, in the form of rapid and marked (depending on the severity of the stenosis) falling-off in diuresis.

In their turn the falling diuresis and the lowered intensity of work of the kidneys entail a reduced oxygen demand of the kidney tissue; this makes possible the appearance of renewed compensatory powers in the kidney, in the form of increase of diuresis. This ability of the kidneys to compensate for disturbance of the diuretic function immediately after its marked diminution may also account for another of our findings – the leveling-off of the diuretic function of the kidneys when the stenosis is not removed for a period of four hours, i.e., throughout the whole experiment. Thus, besides revealing the importance of disturbance of external respiration to the diuretic function of the kidneys, our findings give also some idea of the nature of the compensatory reactions of the kidneys when the intake of oxygen by the body is disturbed.

SUMMARY

Experiments were performed on dogs with ureters lead out under the skin by the I.P. Pavlov-L.A. Orbeli method. The renal urinary excretion was studied under conditions of disturbance of external respiration in tracheal and laryngeal stenoses of variable duration (lasting from several minutes to a few hours). Reaction of the kidney to stenosis depends on the length and severity of the latter, as well as on the functional state of the kidneys when the experiment was started. The peculiarities of the renal compensatory reaction to the disturbance of external respiration were demonstrated. These characteristic features depended on the degree of the functional strain of the kidneys and thus on the oxygen requirement of the renal tissue.

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COMPARISON OF THE EFFECTS OF ISONIAZID DERIVATIVES ON THE DEVELOPMENT OF YOUNG ANIMALS ON A DIET DEFICIENT IN VITAMIN B_6

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Despite the large volume of work done on the synthesis of various derivatives of isonicotinic acid hydrazide (isoniazid), the comparative study of the effects of these compounds on various aspects of metabolism has so far received insufficient attention. We therefore considered that it would be useful to investigate the effect of isoniazid and several of its derivatives (phthivazid, saluzid soluble and metazid*) on vitamin B_6 metabolism. The metabolism of this vitamin was selected as an index of the comparative effect of these preparations on the body because, from reports in the literature, isoniazid has a well-marked effect on vitamin B_6 metabolism.

In about 40% of patients isoniazid causes side effects which are due, in the opinion of several authors, to interference with vitamin B_6 metabolism.

In animal experiments vitamin B_6 prevented the development of polyneuritis and other phenomena which are characteristic of the toxic action of isoniazid [8-11].

In addition to the compounds listed above (phthivazid, saluzid soluble and metazid) we studied acetylated isoniazid and glycuronyl phthivazid, isolated from the urine of rats receiving phthivazid. Both these compounds are of great interest, since they are the detoxication products of phthivazid and isoniazid in the body. For this reason it was interesting to discover how these preparations would act on readministration to the animal.

EXPERIMENTAL METHOD

Acetylated isoniazid is the main conversion product of isoniazid in the body and is far less toxic and 500 times less active than isoniazid [3, 5]. Glycuronyl phthivazid is also the main conversion product of phthivazid in the body, but it retains almost completely the antituberculous activity of phthivazid [1].

Experiments were performed on 76 young male white rats weighing 135-140 g. The rats were kept on a basic diet [2] containing 20% of casein, carbohydrates, fats, mineral substances and vitamins (with the exception of vitamin B_6). To this basic diet were added the following components for the groups of animals (which were kept in cages of six – see scheme).

Rats in all groups not receiving vitamin B_6 received throughout the experiment 10 g of basic diet, and animals receiving vitamin B_6 were given 12 g of food daily, starting on the tenth day of the experiment. The preparations for testing were added to the food in amounts equivalent to 50 mg/kg of isoniazid. The animals were weighed every three days. From 20 to 25 days after the beginning of the experiment the animals were examined

*Isonicotinoyl-(3-methoxy-4-oxybenzal) hydrazone, the diethylammonium salt of isonicotinoyl-(2-carboxy-3,4-dimethoxybenzal) hydrazone and 1-1'-methylene-bis-isonicotinoyl hydrazone respectively – Translator's note.

post-mortem; the blood sugar and hemoglobin were estimated.

EXPERIMENTAL RESULTS

The results of the weight changes of the rats are shown in Table 1 and in Figures 1 and 2; the morphological and biochemical findings in Table 2.

The data in Table 1 suggest that phthivazid and isoniazid inhibit growth of the animals to roughly the same extent. When vitamin B_6 is added to the diet, these preparations have no apparent action (i.e., animals receiving and those not receiving phthivazid and isoniazid developed equally).

Scheme of the Experiments and Composition of the Diets

Group	Number of rats	Diet	
		basic	additions to the basic diet
I	12	basic diet	Without additions – control
II	6	"	+ 50γ vitamin B_6
III	12	"	+ phthivazid
IV	6	"	Phthivazid + 50γ vitamin B_6
V	12	"	+ isoniazid
VI	6	"	+ isoniazid + 50γ vitamin B_6
VII	6	"	+ glycuronyl phthivazid
VIII	4	"	+ acetylated isoniazid
IX	6	"	+ saluzid soluble
X	6	"	+ metazid

All the remaining preparations for testing also caused retardation of growth of the animals. The only noteworthy feature was the more pronounced action of acetylated isoniazid.

TABLE 1

The Effect of Phthivazid and Other Derivatives of Isoniazid on the Growth of Young Rats on a Diet Deficient in Vitamin B_6 (weight of the animals in percentages of the original)

Experimental group	Additions to the basic diet	No. of rats	Day of experiment								
			0	3rd	6th	9th	12th	15th	18th	21st	24th
I	Without additions (control)	12	100	104	105	110	114	118	122	122	125
II	+50γ vitamin B_6	6	100	105	110	111	116	122	131	139	143
III	+ phthivazid	12	100	103	106	108	110	112	115	114	114
IV	phthivazid + 50γ vitamin B_6	6	100	106	109	119	123	128	135	141	145
V	+ isoniazid	12	100	104	107	111	112	113	113	112	113
VI	" + 50γ vitamin B_6	6	100	105	108	112	115	119	127	134	139
VII	+ glycuronyl phthivazid	6	100	103	107	109	112	114	116	117	—
VIII	+ acetylated isoniazid	4	100	103	105	107	109	109	109	110	—
IX	+ saluzid soluble	6	100	103	105	107	109	111	113	111	114
X	+ metazid	6	100	104	107	111	116	117	121	120	119

In their outward appearance the animals of the various groups differed slightly from each other. Rats receiving phthivazid and isoniazid, from the sixth to ninth day became unwilling to eat their food and lethargic, and their fur became dishevelled. In rats receiving saluzid soluble and glycuronyl phthivazid poor appetite was observed after the twelfth to fifteenth day. Rats receiving metazid ate all their food until the eighteenth day of

TABLE 2

Biochemical and Morphological Changes Resulting from the Action of Phthivazid and Other Derivatives of Isoniazid on Rats on a Diet Deficient in Vitamin B_6

Experimental group	Additions to the basic diet	No. of rats	Wt. of rats before killing	Blood hemoglobin (% of normal)	Blood sugar in mg %	Weight of thymus gland		of testicles		of testicular appendages		of adrenals	
						in mg	in % body wt. of animal	in g	in % body wt. of animal	in g	in % body wt. of animal	in mg	in mg %
I	Without addition (control)	10	173	102	115	272	0.16	2.32	1.34	0.64	0.37	30.5	17.5
II	+ 50γ vitamin B_6	6	207	100	125	357	0.17	2.40	1.16	0.92	0.44	33.0	15.0
III	+ phthivazid	10	151	93	116	164	0.11	2.02	1.34	0.49	0.32	30.5	19.5
IV	phthivazid + 50γ vitamin B_6	6	212	101	113	303	0.14	1.99	0.94	1.12	0.53	31.0	15.0
V	+ isoniazid	10	151	91	110	162	0.11	2.15	1.42	0.47	0.31	33.5	22.0
VI	" + 50γ vitamin B_6	6	214	101	119	387	0.18	2.46	1.15	1.02	0.48	32.0	15.0
VII	+ glycuronyl phthivazid	6	160	93	105	230	0.14	2.08	1.30	0.62	0.39	30.0	18.0
VIII	+ acetylated isoniazid	4	145	101	92	242	0.17	2.22	1.53	0.47	0.32	29.0	19.0
IX	+ saluzid soluble	4	149	98	105	155	0.10	2.01	1.35	0.50	0.34	25.0	17.0
X	+ metazid	4	160	97	104	134	0.08	2.35	1.47	0.63	0.39	28.0	18.0

the experiment and looked better than the animals of the other groups. Rats of the control group ate all their food throughout the experiment: outwardly they appeared normal, but they differed from the animals receiving the vitamin by their slow growth and lack of movement.

The results shown in Table 2 show that the blood sugar and hemoglobin were only slightly abnormal; only a slight fall in the hemoglobin content of the blood of rats receiving phthivazid, isoniazid (without vitamin) and glycuronyl phthivazid could be observed, and a tendency for the blood sugar to fall in rats after administration of all the test preparations (and without vitamin B_6).

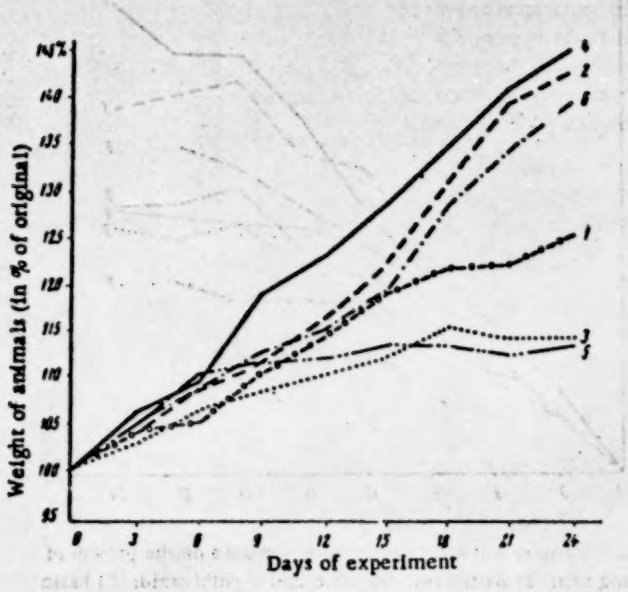

Fig. 1. The effect of phthivazid and isoniazid on the growth of young rats on a diet with and without vitamin B_6: 1) basic diet; 2) basic diet + 50γ vitamin B_6; 3) basic diet + phthivazid; 4) basic diet + phthivazid + 50γ vitamin B_6; 5) basic diet + isoniazid; 6) basic diet + isoniazid + 50γ vitamin B_6.

The characteristic changes found in the organs were diminution of the thymus gland and testicular appendages (in absolute figures and as a percentage of the body weight) and increase in the weight of the adrenals (in percentages of the body weight of the animal). In all the killed animals the thymus gland was prepared, weighed and examined histologically.

Animals of the control group and also of groups IV and VI, which were on a balanced diet containing vitamin B_6 and at the same time received phthivazid or isoniazid did not differ in their outward appearance. All these rats were in a satisfactory nutritional state, the fur was smooth and shining. The average weight of these animals was 210 g. The thymus gland was large in size, milky white in color and of a firm consistency. On microscopic examination no structural changes could be found in the gland in either the control or experimental animals. The lobules of the gland were large and the cortical and medullary layers well defined, with an abundance of cells. The interlobular connective tissue septa were moderately well developed.

Rats kept on a diet lacking in vitamin B_6 and receiving phthivazid, isoniazid, metazid and saluzid were very different in their outward appearance from the rats of groups II, IV and VI. The nutritional state of the animals was depressed; the fur dull and dishevelled. The average weight of these animals was 151.6 g. The thymus gland was greatly reduced in size – less than half the weight of the gland in the control rats. Histological examination showed reduction in the size of the lobules, and narrowing of the cortical zone on account of diminution of the number of lymphoid cells. In the cells of the cortical zone degenerative changes were observed in the form of karyorrhexis or pyknosis. Marked proliferation of the interlobular connective tissue and fatty tissues were observed.

Animals receiving glycuronyl phthivazid and acetylated isoniazid and a diet lacking in vitamin B_6 were identical in weight with the animals of groups III, V, IX and X. The thymus gland of these rats was almost the the same weight as that of the control animals on a diet lacking in vitamin B_6 (group I). Microscopic examination of the thymus gland showed reduction in the size of the lobules, narrowing of the cortical zone on account of a fall in the number of cells, and absence of degenerative changes in the cells of the cortical layer.

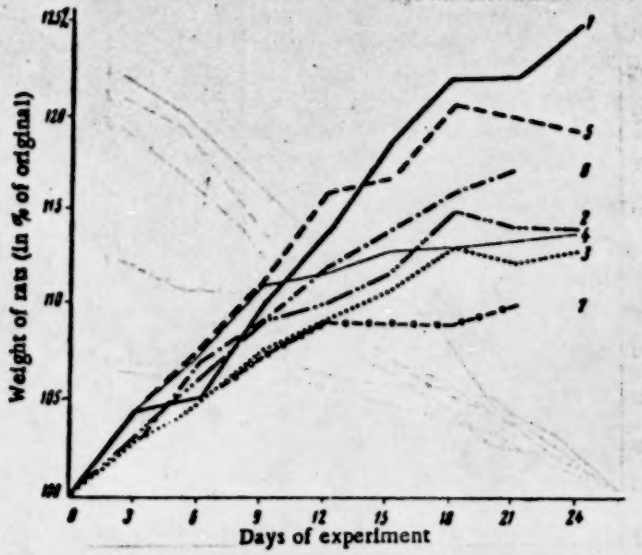

Fig. 2. The effect of derivatives of isoniazid on the growth of young rats: 1) basic diet; 2) basic diet + phthivazid; 3) basic diet + isoniazid; 4) basic diet + saluzid soluble; 5) basic diet + metazid; 6) basic diet + glycuronyl phthivazid; 7) basic diet + acetylated isoniazid.

On the basis of our work it may thus be considered that the effect of all the antituberculosis preparations examined on the young rat is similar in principle and is shown by inhibition of growth and by morphological changes in the organs of the endocrine system – reduction in the weight of the thymus gland and the testicular appendages and increase in the weight of the adrenals. The most pronounced effect on the growth of the animals was shown by isoniazid.

The addition of vitamin B_6 to the diet of the animals removes the inhibiting effect of isoniazid and phthivazid on the growth and the morphological changes in the endocrine system of the experimental animals.

SUMMARY

The effect of the following compounds was studied with regard to their effect on the growth of young rats and on the morphological changes in their organs when kept on a B_6-deficient diet: hydrazide of isonicotinic acid, phthivazid, saluzid soluble, metazid, acetylated hydrazide of isonicotinic acid and glucoronide of phthivazid. All these preparations had an enhancing effect on the signs of B_6-avitaminosis in animals kept on vitamin B_6-deficient diet. The most pronounced effect on the retardation of rats' growth was noted under the action of acetylated HINA, while the least marked action was due to the influence of phthivazid and metazid.

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THE EFFECT OF INTRAVENOUS INJECTIONS OF NOVOCAIN ON THE BLOOD SYSTEM

COMMUNICATION I. CHANGES IN THE COMPOSITION OF THE BLOOD RESULTING FROM REPEATED INJECTIONS OF NOVOCAIN

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In spite of the comparatively wide use of novocain in clinical practice and for experimental purposes, the changes in the blood system resulting from repeated injections of novocain have received quite inadequate study.** According to A. Aslan, addressing the Bucharest congress in 1957, the systematic injection of novocain causes a significant increase in the red and white cell counts of the blood. G.N. Penn [6] proved that after recovery from novocain convulsions the leucocytic reactions were greatly modified.

For two years we have systematically investigated the effect of single and repeated injections of 1% novocain solution on the composition of the blood and bone marrow.

Our findings were obtained from experiments on thirty rabbits and two dogs. In the present paper we give those which relate to the effect of repeated intravenous injections of novocain on the composition of the blood.

All the experimental animals may be divided into three groups. The first contains five rabbits which every day for five to six months received an intravenous injection of 4-5 ml of a 1% solution of novocain (this dose did not usually cause convulsions). The second group contains 14 rabbits and two dogs which were injected every five days for one month, after which the animals underwent a two-stage operation of denervation of the carotid sinuses by a method previously described [7]. After restoration of the composition of the blood the animals were again given novocain in the same doses as before. The third group consists of 11 rabbits in which the changes in the composition of the blood resulting from novocain were studied before and after hemorrhage (the volume of blood withdrawn from the femoral vein varied between 20 and 35 ml). In all cases, control observations on the composition of the blood were made for a period of two to four weeks before the experiment. The bone marrow hemopoiesis was investigated twice (before the start of the novocain injections and one month after them) by means of puncture of the distal epiphysis of the femur. Blood films were stained by Pappenheim's method.

*Deceased.

**We omit here a number of investigations on the effect on the blood system of novocain block [2].

EXPERIMENTAL RESULTS

The results obtained show that injection of novocain may cause, in a portion of the animals, certain ill-defined changes in the composition of the blood. The most characteristic finding was an increase in the hemoglobin content by 11-18 units in 16 out of 30 animals (Figure 1). A reduction in the hemoglobin was observed in one case only, while in the remaining 13 cases the increase in the hemoglobin content was not more than ten units, which is not significant. It began after three to four weeks (but in four rabbits after two weeks), and greatly exceeded the spontaneous variations and changes arising in consequence of injections of physiological saline. In four out of five animals the hemoglobin content remained elevated for three to five months. In rabbit No. 26, for instance, between December 1, 1955 and January 28, 1956 the hemoglobin level varied between 59 and 64 units. On January 29, the novocain injections began. On February 28, the hemoglobin was 78 units and on June 23, 78 units.

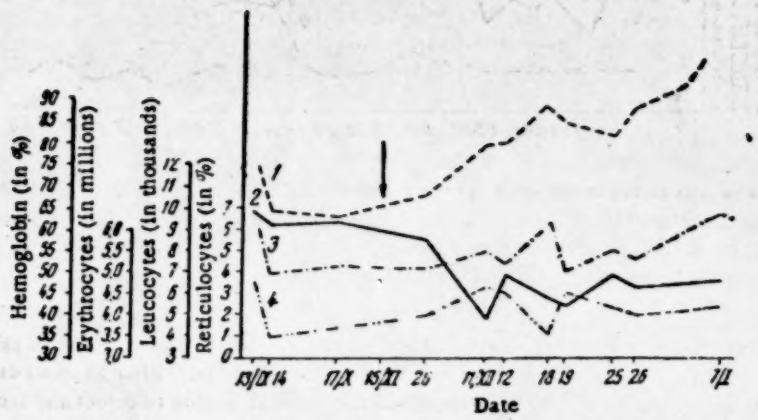

Fig. 1. Changes in the composition of the blood in a rabbit as a result of repeated intravenous injections of 4 ml of a 1% solution of novocain. ↓ – beginning of the novocain injections: 1) hemoglobin content; 2) leucocyte content; 3) erythrocyte count; 4) reticulocyte content.

The mean values of the variations in the hemoglobin content in all 30 rabbits for the month preceding the novocain injections were from +5.2 to −13.8 units, and for the first month of the injections, from +12.2 to −2 units, which undoubtedly shows a tendency for the hemoglobin content of the peripheral blood to rise. In the majority of the animals the erythrocyte count also rose slightly: the mean increase was 580,000 per mm^3 with variations between 300,000 and 1,000,000 per mm^3. In only three out of 30 rabbits was the erythrocyte count lowered by 100,000 to 300,000.

Can it be concluded from these findings that novocain stimulates erythropoiesis? In order to answer this question we studied the reticulocyte content of the peripheral blood of the rabbits. It was found that out of 16 rabbits with a marked increase in the hemoglobin content, in only eight animals was the reticulocyte count raised by more than 1% above its original level. In all the remaining animals it did not vary more than in control animals (Figure 1).

In the bone marrow we counted the cell content of the erythroblastic and leucoblastic series and calculated their ratio. In only nine animals was there observed a slight increase in the number of erythroblasts, mainly orthochromatic. The ratio of leucoblastic to erythroblastic cells before the injections of novocain varied from 2.1:1 to 1.5:1. One month after the first injection of novocain it varied in the same animals from 1.6:1 to 1.1:1. In 21 rabbits, including the seven rabbits with an abnormally high erythrocyte count, no appreciable changes were present in the bone marrow.

Following the novocain injections the leucocyte count rose in 21 out of 30 rabbits, starting on the third week after the first injection. The absolute numbers did not exceed 7000 per mm^3, or 101.3% of the original level. No changes were seen in the leucocytic formula.

The results of the action of novocain on the leucocyte count must be treated with great caution, since rabbits are very prone to spontaneous variations in the numbers of this section of the blood cells, in our experience extending to 103.7% (Figure 2).

In order to shed light on the problem of whether the normal innervation of the carotid sinuses is concerned in the response of the blood system to novocain, we set up special experiments, as already indicated above.

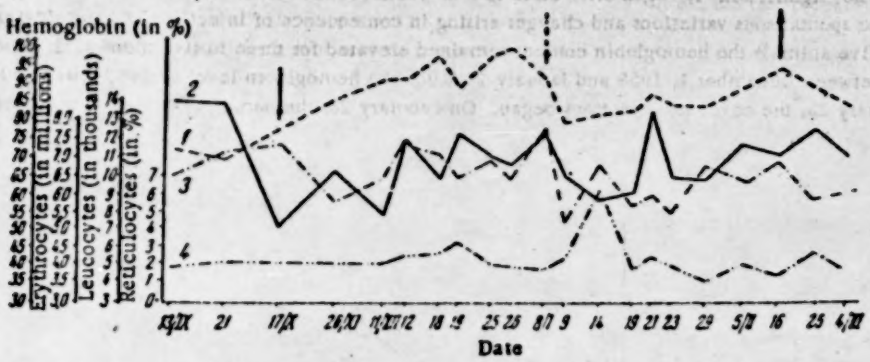

Fig. 2. Changes in the composition of the blood of a rabbit as a result of repeated intravenous injections of 5 ml of a 1% solution of novocain before and after hemorrhage (20 ml). ↓ – beginning of novocain injections; ⇣ – hemorrhage; ↑ – beginning of second course of novocain injections. Remaining signs as in Figure 1.

In these experiments a two-stage denervation of the carotid sinuses was performed on 14 rabbits and two dogs, and when the normal composition of the blood had been restored – usually after 25 to 40 days – a further series of novocain injections was given. In these circumstances we were unable to detect any increase in the hemoglobin and erythrocyte content in one single animal, even after injections lasting for five months, while in two cases a moderate anemia developed.

According to reports by K.V. Maslovskaia [5], N.N. Beller [1], R.A. Durinian [3], denervation of the carotid sinuses leads to a prolonged anemia with a cyclic course. In our experiments 14 of the 16 rabbits did not develop anemia, which must be due to the systematic injection of novocain. These findings are in agreement with those of G.S. Kan and K.A. Poletaeva [4].

Novocain injections were instituted in 11 rabbits after hemorrhage and subsequent restoration of their hemoglobin and erythrocyte levels.

As may be seen in Figure 2, novocain administration in these cases not only did not lead to stimulation of erythropoiesis but in three animals it actually caused a fall in the erythrocyte count, on the average by 640,000 per mm^3, and in the hemoglobin, by 19 units. Consequently, the reaction of the blood system in response to injection of novocain after hemorrhage is essentially modified.

Thus, in our experiments repeated intravenous injections of 4-5 ml of a 1% solution of novocain into rabbits and dogs led to an increase in the erythrocyte count by 540,000 per mm^3 and in the hemoglobin by 11-18 units after two to three weeks in half the animals.

Injection of novocain into animals after hemorrhage and subsequent restoration of the composition of the blood either had no effect on the erythrocyte and hemoglobin values or caused them to be reduced.

SUMMARY

Repeated injection of 4 to 5 ml of 1% novocain solution (once a week) to 30 rabbits resulted in the increase of hemoglobin concentration and the number of erythrocytes. Leucocytosis, without the shift of the leucocytic formula, was also present. No change in the hemopoiesis or in the number of reticulocytes was noted in animals. Injection of novocain to animals with denervated carotid sinuses one month after the operation prevents the development of the first and second wave of anemia in the majority of animals.

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BIOCHEMISTRY AND BIOPHYSICS

THE EFFECT OF SOME LOCAL ANESTHETICS ON PROTEIN METABOLISM IN STRATIFIED SQUAMOUS EPITHELIUM

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Despite many achievements in the field of the pharmacology of local anesthetic drugs, we are still ignorant of the intimate biochemical and physicochemical mechanism of their action. In order to explain the mechanism of local anesthetic action it would be useful to find out how tissue metabolism is affected by local anesthesia. We therefore set out to study the effect of some local anesthetics on protein metabolism in the tissues.

In this work we used two compounds belonging to quite distinct classes of local anesthetic: cocaine and xycain. The latter is new and is identical with the foreign local anesthetic xylocaine. In its chemical structure it is the hydrochloride of diethylamino-2,6-dimethylacetanilide.* According to our experimental and clinical findings and the reports in the literature, xylocaine is distinguished by its low toxicity and high local anesthetic power [4]. In addition, by contrast with cocaine, in its chemical structure it is an amide. It was essential to discover whether any selective action of the different local anesthetics was observed on the intensity of metabolic processes, or whether the local anesthetics act irrespectively of their chemical and physicochemical properties.

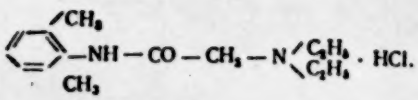
As the subject of the investigation we chose stratified squamous epithelium from the mucous membrane of the tongue of the white rat, which is known to undergo constant and intensive regeneration. This feature makes this tissue very suitable for the study of metabolic relationships.

In solving our problem we employed the method of labelled atoms. An index of the intensity of the metabolic processes going on in the tissues is the quantity of radioisotope taken up by them. In this case we estimated the rate of resynthesis of proteins by the quantity of methionine labelled with radioactive sulfur (S^{35}) taken up by the tissue. Of the methods of working with labelled atoms used in biology, the one which has been most widely used recently is that of autoradiography, which enables not only the intensity of incorporation but also the character of the distribution of the radioisotope in the tissues to be estimated. This makes it possible to relate the metabolic processes occurring in the tissue accurately to its histological structure and thereby to follow the metabolic changes resulting from pharmacological agents.

EXPERIMENTAL METHOD

Experiments were performed on white rats weighing 100-150 g. The animals were starved for 8-10 hours

*Formula of xylocaine:



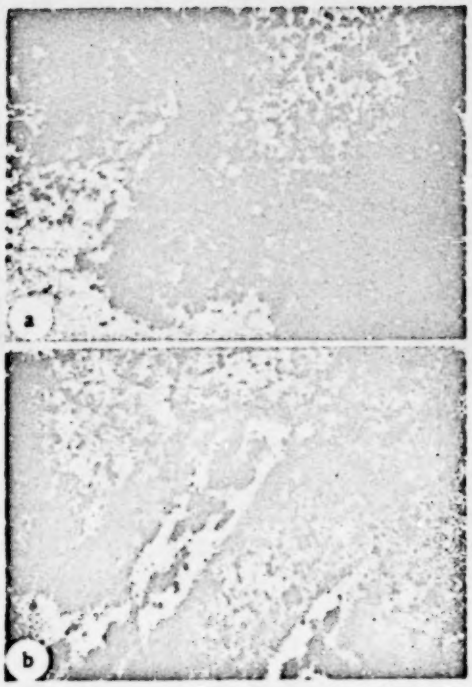


Fig. 1. Tracer autoradiographs of the epithelium of the mucous membrane of the dorsum of the tongue of white rats two hours after injection of methionine labelled with S^{35}: a) normal; b) after local anesthesia. Exposure of photographic emulsion – three days. Objective 60 immersion. Ocular 7.

before the start of the experiment. For the experiment the rats were divided into two groups: experimental and control (with five to six rats in each). Before administration of the radioisotope superficial anesthesia of the mucous membrane of the tongue was carried out in the experimental group of animals by painting it with a 10-20% cocaine solution or with xylocaine. As a rule, anesthesia developed rapidly in both cases and was sufficiently prolonged. Absence of pain in response to stimuli in the form of a prick with a needle served to indicate anesthesia. The mucous membrane of the tongue of the control animals was painted with isotonic saline. Next the animals of both groups were given intravenous injections of methionine, labelled with radioactive sulfur, in a dose of 0.5 μc per 1 g body weight. Two hours after the injection of methionine the rats were decapitated and pieces of the anterior portion of the tongue fixed in Carnoy's solution. After dehydration the material was embedded in paraffin wax and histological sections of a thickness of 7μ prepared from it. On the sections was applied a liquid photographic emulsion by a method described by N.D. Gracheva, L.N. Zhinkin and E.I. Shcherban'.* In the preparation of the substrate and the emulsion, and the developing and fixing of the material, we followed the instructions of the Research Cine-Photo Institute.

Depending on the duration of exposure of the emulsion, trace or contrast autoradiographs were obtained. Autoradiographs were prepared with exposures of 1, 3, 5, 10 and 25 days. Those with exposures of one and three days were trace films – those with exposures of 10 and 25 days – contrast. Exposure of five days was found to be unsatisfactory: the autoradiographs contained too many tracks (traces in the photographic emulsion formed by disintegration of the radioactive sulfur) for a trace film but not enough for a contrast film, i.e., they could not be treated by counting the tracks nor by determining the density of blackening.

The intensity of incorporation of the radioisotope in the tissues was determined from the contrast autoradiographs by the density of blackening of the photographic emulsion on the section, which was measured by a microphotometer MF-4 and registered in units of the logarithmic scale of the microphotometer.** In addition to visual observations and registration of the indications on the microphotometer scale, we recorded simultaneously the density of blackening of the test areas of tissue on a photographic film or plate. On the trace autoradiographs the intensity of incorporation of radioactive sulfur was determined by counting under a microscope with a grid of an ocular micrometer the number of tracks appearing in one square measuring 100 μ^2.

EXPERIMENTAL RESULTS

The rate of incorporation of radioactive sulfur in stratified squamous epithelium of the tongue of white rats in normal conditions was different in the various strata of the epithelium. The most intensive incorporation was found in the cells of the basal germinal layer, especially the basal layer itself. On passing from the basal

*Liquid emulsion type R, issued by the Research Cine-Photo Institute.

**The logarithmic scale of the MF-4 microphotometer is drawn up according to a formula in which the density of blackening is expressed as a logarithmic ratio of the intensity of a beam of light passing through the emulsion outside the preparation to the intensity of a beam of light passing through the area of tissue to be examined.

layer to the surface of the mucous membrane the intensity of incorporation gradually fell and was quite insignificant in the cells of the stratum corneum. The autoradiographic findings on the character of distribution of the radioactive sulfur incorporated in the epithelium of the tongue in rats in normal conditions agree with those reported in the literature [1, 2, 3, and others]. There was a marked change in the intensity of incorporation of radioactive sulfur by the cells of the stratified squamous epithelium of the tongue during local anesthesia. In the experimental animals the incorporation of methionine labelled with radioactive sulfur took place more slowly than in the controls.

The table shows the mean results of the track counts for ten squares of the ocular micrometer grid, i.e., for 1000 μ^2, taken from the 100 counting units (squares) and the mean results of photometry obtained from ten (for each exposure) contrast autoradiographs.

Change in the Intensity of Incorporation of Radioactive Sulfur in the Cells of the Stratified Squamous Epithelium of the Mucous Membrane of the Tongue in White Rats as a Result of Local Anesthesia

Epithelial layer*	Average number of tracks per 1000 μ^2 (trace autoradiographs)						Average density of blackening in units of the logarithmic scale of the MF-4 microphotometer (contrast autoradiographs)					
	1 day			3 days			10 days			25 days		
	control	expt.	expt.** / control (%)	control	expt.	expt. / control (%)	control	expt.	expt. / control (%)	control	expt.	expt. / control (%)
First layer	16.4	9.0	54.9	32.7	19.0	58.1	34	23	67.6	67	48	71.6
Second layer	17.0	8.3	48.8	29.6	17.2	58.1	24	16	66.6	52	36	69.2

*The first layer corresponds histologically to the basal portion of the stratum germinativum, i.e., to the cells of the basal and prickle-cell layers; the second layer embraces the cells of the prickle-cell layer and the adjacent stratum granulosum.

**$\frac{\text{Experiment}}{\text{control}}$ (%) is the ratio between the results in the experimental and control animals expressed as a percentage.

The difference in the intensity of incorporation of radioactive sulfur which we observed between the control and experimental groups was shown in both photometry and track counts. If we take the intensity of incorporation in the control animals to be 100%, in the experimental animals, according to the results of track counts, it was reduced in various layers to 48.8-58.9%, and, according to photometry, to 66.6-71.6%.

As an illustration of the above, in Figure 1 are shown photographs of trace microautoradiographs of control (a) and experimental (b) animals – even with the naked eye the difference in the intensity of incorporation of radioactive sulfur is obvious. Similar findings were obtained by photographic recording of the density of blackening of contrast microautoradiographs by means of a MF-4 microphotometer (Figure 2). It is clear that the density of blackening in the control (a) reaches 75 divisions of the logarithmic scale of the microphotometer, while in the experiment (b) it hardly reaches 40. Comparing the findings obtained by photometry with those obtained from track counts, we see that they are in complete agreement.

No essential difference between the intensity of incorporation in the experimental groups could be detected: it appeared that xylocaine and cocaine have an equal action on the intensity of the metabolic processes, slowing the rate of resynthesis of proteins to about the same degree.

On the basis of the experiments reported in this paper it can be concluded that our method of autoradiography enables us to judge the character of the changes arising in certain cell strata, and also their intensity. In studying the effect of local anesthesia in the tissue metabolism we have proved that the intensity of the metabolic processes taking place in the epithelium of the tongue is diminished as a result of local anesthesia. However the metabolic relations observed in normal animals are preserved.

It is my pleasant duty to express my profound thanks to Prof. L.N. Zhinkin and to my colleagues in the laboratory of histology, under his direction, at the Institute of Experimental Medicine of the Acad. Med. Sci. USSR

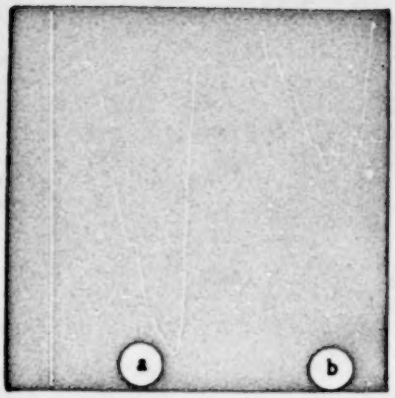


Fig. 2. Photometric curves of contrast autoradiographs obtained by photographic recording on a MF-4 microphotometer two hours after injection of methionine labelled with sulfur (S^{35}): a) normal; b) during local anesthesia. Exposure of the emulsion – 25 days. Along the vertical axis are shown the divisions of the logarithmic scale of the microphotometer (explanation in text).

for frequent advice and help during the course of this work.

SUMMARY

A study was carried out to discover the effect of local anesthesia on the intensity of incorporation of S^{35}-labelled methionine into the cells of stratified epithelium of the tongue mucosa in white rats. It was established that the velocity of incorporation of S^{35} into the cells of stratified epithelium is retarded under the effect of local anesthesia (10-20% xycaine or cocaine). Consequently, the velocity of protein resynthesis in this tissue is also retarded. However, the character of distribution of S^{35} in various layers of the epithelium is not changed. No pronounced difference was noted in the intensity of incorporation of S^{35} in administration of cocaine or xycaine. It was demonstrated that both anesthetics affect the intensity of metabolism in the same way, and retard the resynthesis of protein to about the same degree.

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THE EFFECT OF GLUTAMIC ACID ON THE OXYGEN DEMAND OF ANIMALS IN A STATE OF ANOXIA

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According to numerous reports in the literature [3], the addition of glutamic acid to pulp and extracts of various tissues increases their oxygen demand. However, there is little reference in the literature to the problem of the effect of administration of glutaminic acid on the oxygen demand of the animal as a whole.

Nevertheless, in recent years glutamic acid and its salts have become quite extensively used in the treatment of the most varied diseases, some of which are undoubtedly accompanied by some degree of anoxia, for example, cerebral vascular disorders in the newborn [1, 5], cardiac failure in adults [10], the state of clinical death [6, 7], and so on.

In connection with these findings it is useful to investigate the effect of glutamic acid on the oxygen demand of animals when the inspired air is normal or is deficient in oxygen.

EXPERIMENTAL METHOD

Experiments were performed on young white rats. For measuring the oxygen demand a specially constructed apparatus was used which ensured absorption of the expired carbon dioxide, constancy of a predetermined concentration of oxygen and control over its demand for any required period of time. Construction of the apparatus al-

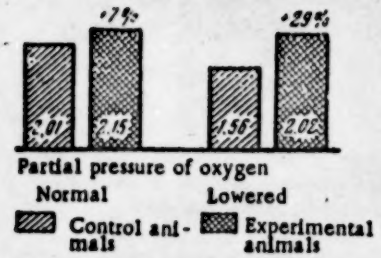

Fig. 1. The effect of administration of sodium glutamate on the oxygen demand of rats (mean results) in ml of oxygen per g body weight per hour.

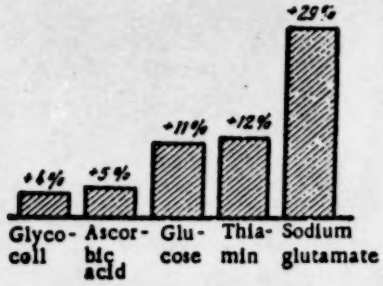

Fig. 2. The effect of administration of various compounds on the oxygen demand of rats (mean results). The values are given in percentage increase of oxygen demand (in ml of oxygen per g body weight per hour) of the experimental animals compared to the controls.

lows simultaneous observations to be carried out on two rats kept under identical experimental conditions but isolated from each other.

Two starving rats were placed in the apparatus for 1½ hours to become accustomed to the conditions of the experiment. Next, for a period of one hour their oxygen demand was determined every five minutes. After this, the system was filled with a mixture of nitrogen (78%) and oxygen (12%), and under these conditions the oxygen demand was again determined in the same way for a further hour. Next, one of the rats was injected subcutaneously with sodium glutamate (1 mg per 1 g body weight) in 5% solution. The control rat was injected with the same volume of physiological saline. Afterwards the oxygen demand under conditions of anoxia was again determined for a period of 2-4 hours.

By way of control observations a series of experiments was performed in which the effect of sodium glutamate on the oxygen demand of rats could be examined with a normal partial pressure of oxygen.

EXPERIMENTAL RESULTS

When the partial pressure of oxygen in the inspired air is normal the injection of sodium glutamate into rats causes no perceptible change in their oxygen demand in comparison with rats not given glutamate. In similar experiments when the oxygen content of the inspired air was reduced, the oxygen demand was very greatly increased after injection of glutamate. Its increase during a two-hour experiment amounted to 29% on the average over its value in control animals (Figure 1).

If, instead of glutamate, the same dose of glycocoll or glucose, or ascorbic acid (20 mg) or thiamine (1 mg) was injected into rats kept in an anoxic state, only a slight increase in the oxygen demand compared with control animals was produced (Figure 2).

Sodium glutamate thus possesses the specific property of considerably increasing the oxygen demand of animals kept in a state of anoxia.

It is not yet possible to give a complete analysis of the mechanism by which glutamic acid stimulates oxidative processes during anoxia. It may be that glutamic acid acts by increasing the synthesis of hemoglobin from pyrrolidonecarbonic acid [8], and we consider that a leading part in this process is played by activation of the reactions of the tricarboxylic acid cycle.

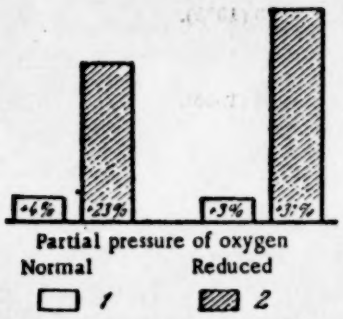

Fig. 3. The effect of injection of lactic and glutamic acid on the oxygen demand of rats (mean results). Values given in percentages of increase of oxygen demand (in ml per g body weight per hour) of the experimental animals over the controls: 1) injection of lactic acid; 2) injection of lactic and glutamic acids together.

Injection of glutamic acid into an animal is known to lead to an increase in the α-ketoglutaric acid content of the tissues and blood [9, 11]. This compound, undergoing further oxidation to succinic and other dicarboxylic acids, results in an increase in the oxalacetic acid concentration, which creates favorable conditions for involving lactic acid and other incompletely oxidized products in the tricarboxylic acid cycle. This is confirmed by the findings of Braunshtein and Kritsman [2, 4] that glutamic acid leads to a decrease in the lactic acid content during aerobiosis. It was to be expected that injection of glutamic acid, with a normal partial pressure of oxygen and with not too great a concentration of incompletely oxidized products, in particular lactic acid, would not have led to a significant increase in the oxygen demand, as in fact took place (see Figure 1).

In anoxic conditions the content of lactic acid and other incompletely oxidized products in the animal tissues increases sharply, and therefore the injection of glutamic acid must facilitate their complete oxidation, thereby leading to an increase in the oxygen demand.

In order to test the correctness of this hypothesis a series of experiments was performed to examine the oxygen demand of rats after preliminary injections of lactic and glutamic acid.

It was found that injection of lactic acid did not cause any increase in the oxygen demand during normal partial pressure of oxygen or anoxia. Injection of lactic acid together with glutamic acid led to

a considerable increase in the oxygen demand, more pronounced during anoxia (Figure 3).

Thus, the results of this series of experiments show that glutamic acid stimulates the oxidation processes not only in tissue pulp and extracts, but also in the intact animal.

The results of the experiments which we have described give reason to suppose that stimulation of the oxygen demand by glutamic acid during anoxia is undoubtedly due to the increased involvement of incompletely oxidized metabolic products in the oxidation cycle of the tricarboxylic acids.

SUMMARY

The effect of subcutaneous introduction of glutamic acid on the oxygen intake of rats was investigated in conditions of normal and decreased (12%) content of oxygen in inhaled air. It was established that the introduction of glutamic acid in normal partial oxygen pressure causes only insignificant increase of its intake (by 6%). In condition of hypoxia it is increased by 29%. On the basis of experiments it is suggested that the stimulating effect of glutamic acid is the result of its ability to introduce the products of metabolism which are not fully oxidized into the oxidative cycle of tricarboxylic acids.

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THE IMPORTANCE OF MITOGENETIC RAYS IN THE SYNTHESIS OF PEPTIDES IN THE LIVER

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In 1937-1938 A.G. and L.D. Gurvich [1, 9] showed that during irradiation of mixed solutions of amino acids with mitogenetic rays, compounds of relatively high molecular weight were formed, which, on digestion with gastric juice, produced mitogenic rays of a spectral composition identical with that characteristic of the peptide bond.

This work was of great importance in principle since it showed the possibility of in vitro synthesis of biologically important compounds by a photochemical rather than an enzymic method. These workers put forward the hypothesis that in the living organism, thanks to the mitogenetic system, peptide synthesis may take place by a photochemical method in addition to and without the participation of enzymes.

A.G. and L.D. Gurvich [3] further showed the identity of the energy requirements for stimulation of peptide synthesis and cellular division by mitogenetic rays: both peptide synthesis and cell division can be brought about by any wavelength of the mitogenetic spectrum from 1900 to 2700 A for irradiation in darkness and to 3260 A for irradiation in light or in infrared radiation. This convinced these workers of the great biological importance of the fact that the mitogenetic action of ultraviolet light begins with stimulation of peptide synthesis.

It must be pointed out that suppression of mitogenetic rays by the administration of a tumor-destroying agent or of extinguishers of mitogenetic rays in living objects leads to a sharp fall in the number of dividing cells [3, 4] and at the same time to marked vacuolization of the protoplasm of the majority of even the young cells while in many cases the whole body of the cell appears as one continuous vacuole. This condition, analogous to that observed in the rootlets of bean plants whose cotyledons have been cut off, is usually attributed to protein starvation of the cells.

In 1945 A.G. and L.D. Gurvich [10] introduced a new method of mitogenetic spectral analysis with selective dispersion, making it possible to compile an atlas of the characteristic spectra of functional molecular groups or, in some cases, of whole molecules of various organic compounds.

Because of its sensitivity, this method permits the analysis of extremely transient processes taking place in vitro and in vivo as rare events. By means of this method E.A. Ternovskaia (cited in [11]) obtained more direct experimental proof of peptide synthesis by mitogenetic irradiation of amino acid mixtures.

In 1953-1954 A.G. and A.A. Gurvich (cited in [11]) showed that in the dark, peptide synthesis in the leaves of green plants takes place less intensively than in the light, since only in the light do mitogenetic rays arise in them (according to N.M. Peredel'skaia [7]) in consequence of summation of the energy of the photons of visible light to the level of ultraviolet radiation. In plants kept in the dark, peptide synthesis may be stimulated by irradiation with mitogenetic rays, but in plants grown in the light it is inhibited by suppression of mitogenetic rays

by means of injection of the so-called extinguishers.

During prolonged action of extinguishers the diminution in protein synthesis is so considerable that growth of the experimental plants is retarded in comparison with controls. These results obtained by A.G. and A.A. Gurvich (cited in [11]) confirm the necessity of mitogenetic rays for peptide synthesis in the green leaves of plants.

In 1934 A.G. and L.D. Gurvich [2] found that in contrast to all other organs the liver and kidneys of animals on a normal diet do not produce mitogenetic rays. A.G. Gurvich expressed the view that as a result of the fact that processes of syntheses were predominant in the liver, it contains only an insignificant amount of the so-called fluorescents – low molecular compounds able to emit energy in the form of rays. High molecular compounds – proteins, glycogen and so on – do not fluoresce. Hence it followed in the first place that the liver of fasting animals in which processes of breakdown are predominant may possibly emit ultraviolet light, and in the second place that injection of fluorescents into normally fed animals should lead to emission of rays from their livers. Both these conclusions derived from A.G. Gurvich's hypothesis have been confirmed experimentally in work by E.A. Ternovskaia and V.F. Eremeev (cited in [11]).

The results of the investigations by E.A. Ternovskaia and V.F. Eremeev, and also reports in the literature on the synthesis of proteins in the liver of normally fed animals and of protein breakdown in the liver of fasting animals have enabled the study of the importance of mitogenetic rays in peptide synthesis in the liver to be undertaken.

It must be pointed out that the majority of authors [5, 6, 12, 13, 14, 15, 16] consider that the liver plays a great part in synthesis during protein metabolism, and assume that the liver has the power to accumulate proteins which are mobilized to meet the needs of the body during protein starvation more rapidly than proteins of other organs (especially in the first stages of starvation).

In this connection it might be supposed that in early stages of an animal's starvation the breakdown processes of protein substrate are still so ill-developed that the quantity of fluorescents formed in this way is not sufficient to reveal even threshold intensities of mitogenetic radiation. At the same time there is quite enough of them to reveal breakdown processes of protein substrate by the method of spectral analysis with selective dispersion.

If this hypothesis of A.G. Gurvich could be confirmed experimentally, an attempt might be made to irradiate the liver externally from some mitogenetic source and cause partial resynthesis of the protein substrate, which could be judged by the threshold exposures necessary to show selective dispersion of NH_2- and OH-groups, the amounts of which are increased during breakdown of protein substrate and decreased during its resynthesis. This investigation forms the content of the present paper.

EXPERIMENTAL METHOD

White mice were kept for 18 to 20 hours on a fixed diet (millet, white bread and milk). An excess of food was given to the animals. At the conclusion of this period they were totally fasted. Fasting lasted from 3 hours 30 minutes to 5 hours 30 minutes.

It was first established that radiation appeared in the liver during starvation of the animal for 5 to 5½ hours.

Further experiments were performed in accordance with the following scheme. After fasting for four hours it was checked whether there was spontaneous radiation in the liver of the animal, and the selective dispersion spectrum of the amino groups was taken. Next the liver was irradiated for 15-30 minutes with mitogenetic rays from a source of radiation and the selective dispersion spectrum of the amino groups of the protein substrate again taken at different exposures. In this way, in the first series of experiments the selective dispersion spectrum was taken from the same aspect (ventral) as the lobe of the liver irradiated; in the second series of experiments, from the opposite aspect, i.e., the liver was irradiated on the dorsal aspect and the spectrum taken from the ventral aspect.

In order to obtain full confirmation that all the manipulations carried out on the liver were not affecting its condition, special control experiments were set up in which everything was done to the liver just as has been described, but it was not irradiated from a source of mitogenetic rays.

EXPERIMENTAL RESULTS

The results of all the experiments are shown in the table.

It follows from the table that spontaneous emission of rays does not occur with fasting for four to five hours, and the amino groups of the protein substrate of the liver can be revealed by their selective dispersion at exposures of 10-12 seconds and that after mitogenetic irradiation of the liver these amino groups cannot be detected, not only at exposures of 10-20 seconds, but also of 15, 20 and 25 seconds, and they are demonstrable only at exposures exceeding 30 seconds; furthermore the same results are found when the liver is irradiated on its dorsal aspect and the spectrum taken from its ventral aspect. The manipulations carried out on the liver do not affect its condition in any way, as shown by experiments in which no irradiation was carried out and the amino groups of its protein substrate were revealed at exposures of ten seconds, as also occurred without manipulations after the same period of fasting.

Mitogenetic Spectra of Terminal NH_2-Groups of Peptides in the Liver of Fasting Mice Before and After Irradiation

Period of fasting	Exposure (in seconds)	Spectral band 2060-2070 A. Mean effect (in %)	Spectral band 2260-2270 A. Mean effect (in %)	Number of experiments
Spontaneous radiation from the liver of fasting mice				
3 hours 30 minutes to 5 hours	10-15	1.0	1.3	18
5 hours 30 minutes	10	40.3	58.0	3
Selective dispersion of the liver of fasting mice				
4 hours 10 minutes to 5 hours 25 minutes	10-12	34.3	33.9	18
Selective dispersion of the liver of fasting mice after irradiation from a source of mitogenetic rays for 15-30 minutes				
4 hours 20 minutes to 5 hours 40 minutes	12	0	2	5
	15	−1	0	4
	20	−1	1	4
	25	0	2	3
	30	37	32	3
Selective dispersion of the liver of fasting mice after performing all steps of the experiment except mitogenetic irradiation				
4 hours 50 minutes to 5 hours	10	27.3	44.0	3

The results obtained show that in the liver of mice at early stages of fasting (four to five hours) processes have already begun which can be detected by the method of spectral analysis of selective dispersion. Increase in the intensity of these processes leads to the appearance of spontaneous emission of rays by the liver after fasting for five hours.

Irradiation of the liver of fasting mice from a mitogenetic source increases the threshold exposures necessary for detecting the terminal amino groups, which proves that they have diminished in quantity and is accounted for by the partial resynthesis of the protein substrate of the liver tissue.

The discovery of a reduction in the quantity of terminal amino groups during irradiation of the liver on its dorsal aspect and taking the spectrum from its ventral aspect shows that the processes of partial resynthesis of the protein substrate as a result of mitogenetic irradiation have a chain character and involve the whole thickness of the liver.

It thus follows from the results of the work described that mitogenetic rays stimulate peptide synthesis in the liver of animals.

How does peptide synthesis take place in the normal liver which does not emit rays but which acquires the power of radiation when fluorescents are administered to it? In order to answer this question it is necessary to remember how mitogenetic rays arise during enzymic reactions: the large quanta of energy given out during recombination of free radicals or atoms, arising as rare phenomena in enzymic processes, are absorbed by neighboring molecules which then emit the absorbed energy in the form of mitogenetic rays.

Since fluorescents (molecules emitting energy in the form of rays) are present in the liver in small concentrations, photons of mitogenetic rays arise so rarely that radiation cannot be found even by biological detectors, but nevertheless peptide synthesis may take place at subthreshold intensity.

Another factor stimulating synthesis of peptides may probably be the above-mentioned quanta of energy, which are emitted quite irrespective of whether there is an adequate concentration of fluorescents.

These considerations just mentioned lead us to the hypothesis that the liver of animals which are affected by malignant neoplasms, containing a cancerous suppressor of mitogenetic radiation preventing the formation of large quanta of energy, is, despite the influx of all necessary food substances, in a state of protein starvation to some degree, in consequence of the absence of the necessary energy conditions for peptide synthesis.

The experimental verification of this hypothesis will be dealt with in a special paper.

SUMMARY

The first traces of disintegration of the protein substrate of the liver (in situ) were revealed in hungry animals by the method of mitogenetic spectral analysis of selective scattering of the ultraviolet radiation when the animals were fasted for four to five hours. Resynthesis of the protein substrate of the liver may be caused by irradiation of the liver with the nitrogenetic source of radiation during these "hungry" periods. Thus, stimulation of the peptide synthesis by the mitogenetic radiation may be demonstrated.

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PHARMACOLOGY

THE ANTAGONISTS OF AMINAZINE IN RELATION TO ITS HYPOTENSIVE AND ADRENOLYTIC ACTION

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Because of the widespread clinical use of aminazine, and its side-effects which are especially obvious with overdoses, the question of antagonists or "correctors" of this drug acquires great practical significance. The literature lists caffeine, theophylline and euphylline [2] as antagonists of aminazine. However, it still has not been established whether these substances antagonize all the aminazine effects or only several.

One of the factors limiting the use of aminazine is its property of inducing a prolonged depression of arterial pressure [1, 5]. The danger of such an effect is increased by the fact that, as one of its other properties, it has an adrenolytic effect so that the attempt to employ adrenalin and other such pressor substances against this aminazine background can lead to perverted effects.

There have been experimental studies [3, 4] devoted to the investigation of the hypotensive actions of aminazine and the use of substances raising the arterial pressure depressed by aminazine. These authors included among such substances noradrenalin, mesaton, phenamine and pervitin. However, the findings of these authors are contradictory, diametrically opposing statements being made at times. Thus, for example, Krause and Schmidtke-Ruhnan, studying the sympatholytic action of megaphen (aminazine) stated that the arterial pressure depressed by megaphen administration can be raised with phenamine or pervitin. Marquardt and his co-workers indicated [4] that phenamine and pervitin not only did not raise the arterial pressure depressed by megaphen but that there is actually a perversion of their normal action.

For all these reasons we believed it advisable to search for antagonists of the hypotensive and adrenolytic effects of aminazine.

EXPERIMENTAL METHOD

The experiments were performed on cats under urethane, chloralose or nembutal narcosis as well as on decerebrate cats and intact rabbits. The arterial pressures were measured with a mercury manometer connected with the carotid artery. Respirations were recorded with the aid of a Marey tambour connected with a tracheal cannula.

The adrenolytic action of the aminazine was determined using Marquardt's method. Before beginning the experiment, the minimal intravenous pressor dose of adrenalin was determined. As a rule, this was 5-20 μg of adrenalin. After the introduction of aminazine, because of its adrenolytic effect, this dose of adrenalin no longer produced a pressor effect. We recognized two degrees of adrenolysis: in the first stage, the injection of adrenalin failed to produce a pressor effect; in the second, adrenalin injection led to a definite depressor effect. This method was most suitable for the purposes of the present investigation as the difficulties in elevating the arterial

pressure depressed by aminazine arise in the first and especially in the second stage of adrenolysis.

Aminazine was employed in intravenous doses of 0.5 to 35 mg/kg.

As possible aminazine antagonists we studied the following: noradrenalin (5-20 μg), mesaton (10-15 μg), ephedrine (1-5 mg/kg), phenamine (1-2 mg/kg), pervitin (2 mg/kg), corazole (2 mg/kg), cordiamine (10-15 mg/kg), strychnine (0.1 mg/kg), caffeine (0.1 mg/kg), picrotoxin (0.2-0.45 mg/kg), lobeline (0.25-0.30 mg/kg), euphylline (20 mg/kg), β-tetrahydro-naphthylamine (0.5-15 mg/kg), pituitrin (0.1-1 units/kg), pyrogenal (10-20 units), as well as 10-allyl-phenothiazine and 10-allyl-2-chlorphenothiazine (5-10 mg/kg).

All the substances were introduced intravenously, the only exception being the insoluble allyl derivatives of phenothiazine which had to be given intra-abdominally as part of a starch suspension.

EXPERIMENTAL RESULTS

a) Search for Antagonists of the Hypotensive Effect of Aminazine

In the experiments on cats under urethane and nembutal narcosis as well as on the decerebrate cats and intact rabbits, it was established that of all the tested substances only noradrenalin and pituitrin are capable of elevating the blood pressure depressed by aminazine. Noradrenalin against an aminazine background is almost as effective as before aminazine, while adrenalin after aminazine does not show a pressor effect (Figure 1a).

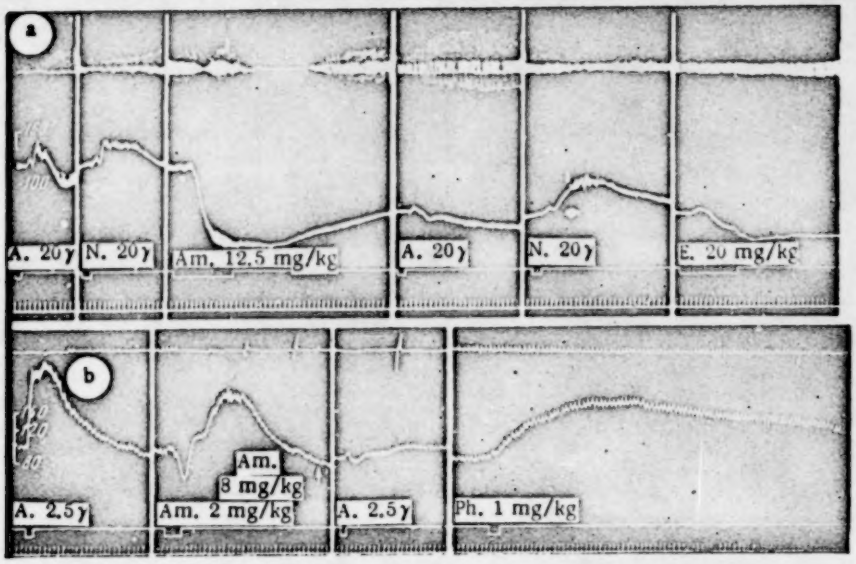

Fig. 1. Pressor action of noradrenalin and phenamine against the background of aminazine. Significance of curves (from above down): respiration; application of stimulus; A) introduction of adrenalin; N.A.) introduction of noradrenalin; Am) introduction of aminazine; E) introduction of euphylline; Ph) introduction of phenamine; a) under urethane narcosis; b) under chloralose narcosis. Time marker – five seconds.

The action of noradrenalin is relatively fleeting. To prolong the pressor effect of noradrenalin, it should be introduced by the drop method. At times, the pituitrin effect is re-enforced and prolonged by the antecedent injection of aminazine. This is especially noticeable when pituitrin is introduced by the drop method (Figure 2). Before the introduction of aminazine, the drop injection of 1 unit/kg of pituitrin in 10 ml of physiological saline over an eight-minute period produces only an insignificant rise in arterial pressure while, after the introduction of aminazine, even against a second degree adrenolytic effect, the same amount of pituitrin introduced in a similar manner produces a marked, almost instantaneous arterial rise (in order to avoid manifestations of tachyphylaxis, the second pituitrin injection was not given until at least 1½ hours after the first).

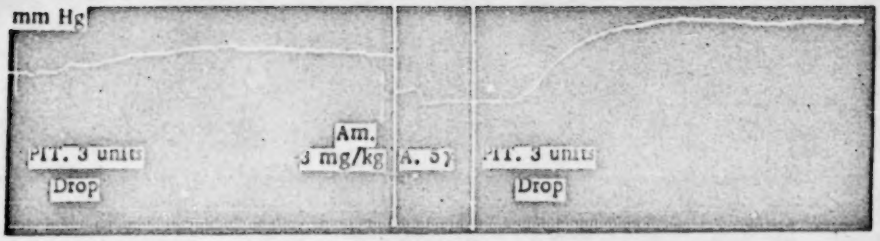

Fig. 2. Pressor action of pituitrin against an aminazine background. Significance of curves (from above down): blood pressure, stimulus marker; P) drop introduction of pituitrin (1 unit to 1 kg weight in 10 ml of physiological saline for eight minutes); Am) introduction of aminazine; A) introduction of adrenalin. Time marker – five seconds.

When the animals received larger doses of pituitrin (1 unit/kg) coronary spasm developed with upsetting of the cardiac rhythm which had to be restored with the use of papaverine.

The other substances, tested in the doses indicated, produced uncertain effects. For example, cordiamine in some instances produced insignificant fleeting arterial pressure rises, while, in other instances, the pressure either did not rise or actually became depressed.

Euphylline (Figure 1a) and lobeline against a background of aminazine action, as a rule gave markedly pronounced depressor effects.

Thus, the antagonists to the hypotensive action of aminazine are noradrenaline and pituitrin. At this point it should be repeated that our results were obtained in experiments on cats under urethane and nembutal narcosis as well as on decerebrate cats and intact rabbits.

During the course of our experiments we showed that chloralose narcosis materially alters results, for, in order to obtain adrenolysis by the use of aminazine under chloralose narcosis, quite large doses had to be employed so that second degree adrenolysis occurred much less often. In addition, chloralose narcosis muted the hypotensive effects of aminazine markedly. Even the arterial pressure reaction in response to aminazine introduction under chloralose narcosis is different from that observed when other anesthesia methods are employed. Introduction of aminazine under this type of narcosis results in an arterial pressure rise succeeding a fleeting depression (Figure 1b). In experiments conducted under urethane narcosis introduction of aminazine produces an immediate profound depression of the arterial pressure (Figure 1a).

In the experiments conducted under chloralose narcosis the arterial pressure, depressed by aminazine, rises after the introduction of noradrenalin and pituitrin just as it does in the experiments employing other methods of narcosis.

In addition to these substances, phenamine is capable of elevating the arterial pressure in experiments conducted under chloralose narcosis. Phenamine in doses of 1-2 mg/kg (Figure 1b). introduced after aminazine, results in a considerable, prolonged rise of pressure, a phenomenon not obtained with phenamine when other methods of narcotization are used.

b) Search for Antagonists of the Adrenolytic Effect of Aminazine

It is more difficult to remove the adrenolytic effect of aminazine than it is to remove the hypotensive action. Of all the substances examined ephedrine only,and then for a short time and with chloralose narcosis only, would restore the pressor reaction of adrenalin (Figure 3a). With time the pressor response to adrenalin continued to diminish so that within 10-15 minutes adrenolysis occurred again. However, another injection of ephedrine restored the pressor response to adrenalin. With other narcotizing agents and in intact rabbits ephedrine failed to produce such an action.

The special characteristics of these experiments under chloralose narcosis made us believe that chloralose

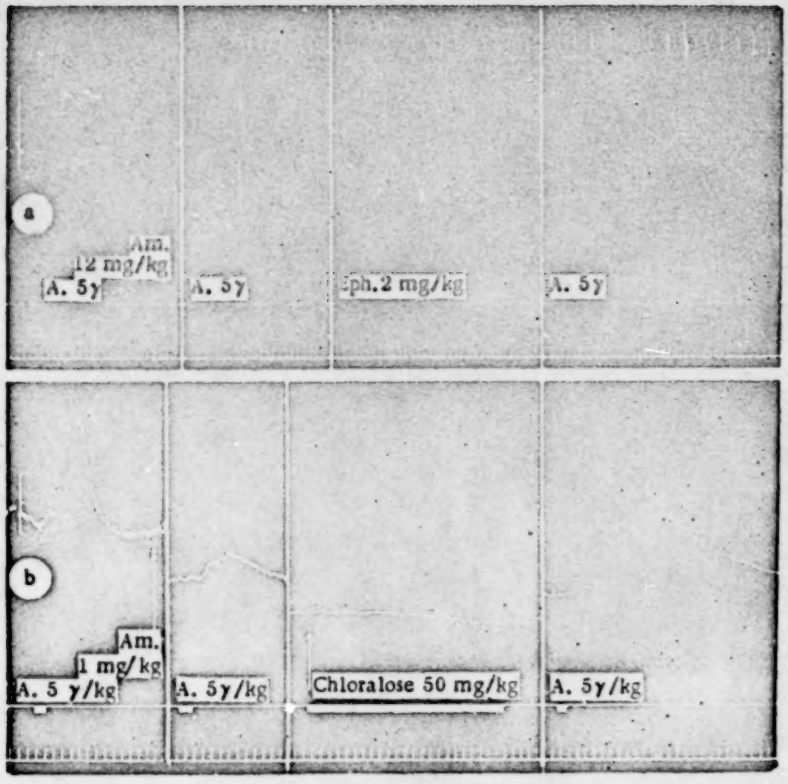

Fig. 3. Restoration of the pressor reaction of adrenalin by the use of ephedrine and chloralose against a background of aminazine. Significance of curves (from above down): blood pressure, stimulus marker; A) introduction of adrenalin; Am) introduction of aminazine; Eph) introduction of ephedrine; a) chloralose narcosis; b) with decerebration. Time marker – five seconds.

itself might have an influence on the adrenolytic properties of aminazine.

In order to answer this question, we did a few experiments on cats under urethane and ether narcosis as well as on decerebrate cats and used chloralose as a possible aminazine antagonist.

After giving the animals aminazine and obtaining adrenolysis, we injected chloralose intravenously in doses of from 50 to 100 mg/kg but this failed to produce arterial pressure levels. In cases in which the aminazine dose had been as small as 1 mg/kg, the pressor response to adrenalin was temporarily restored to almost the initial levels (Figure 3b).

It appears that, on the basis of our experiments, the antagonists of the hypotensive action of aminazine are noradrenalin and pituitrin.

Under conditions of chloralose narcosis, phenamine is also an antagonist of aminazine. Ephedrine is an antagonist of the adrenolytic effect of aminazine only when chloralose narcosis has been used. Chloralose by itself is not capable of decreasing the adrenolytic action of aminazine.

Arterial pressure, depressed by aminazine, may be raised by the introduction of noradrenalin and pituitrin. The drop introduction of these substances seems the most practical method.

Practically speaking, not one of the substances tested removed the adrenolytic effect of aminazine.

The conflicting opinions of various investigators working with aminazine antagonists are explained by their use of different narcotizing agents.

SUMMARY

Acute experiments were performed on cats anesthetized by different methods, as well as on intact rabbits. Noradrenalin, mesaton, phenamine, picrotoxin, lobeline, euphylline, β-tetrahydronaphthylamine, pituitrin, pyrogenal, allyl-phenothiazine, allyl-2-chlorphenothiazine were tested as possible antagonists of the hypotensive and adrenolytic effect of aminazine. Only pituitrin and noradrenalin caused the rise of arterial blood pressure, decreased by aminazine. Phenamine had the same effect as these two substances in experiments performed on cats under chloralose anesthesia. Adrenolytic effect of aminazine could be removed by injection of ephedrine only in experiments where chloralose anesthesia was employed. In connection with the fact that chloralose anesthesia had a pronounced effect on the pharmacological properties of aminazine, chloralose was tested as a possible antagonist of aminazine. It appeared that it decreases the adrenolytic effect of aminazine but slightly.

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THE BLOCKING EFFECT OF CHOLINOLYTIC SUBSTANCES AT THE PARASYMPATHETIC GANGLIA*

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In the pharmacological investigations of cholinolytic medicinals, their blocking effects are of considerable interest. To study the effect of cholinolytic substances upon sympathetic ganglia is a relatively simple matter; to do the same with parasympathetic ganglia is a much more difficult procedure. One of the difficulties lies in the fact that the parasympathetic postganglionic fibers are very short. This anatomic peculiarity practically excludes perfusion of the parasympathetic ganglia, even when this method is the most satisfactory one known for studying the ganglionic actions of various substances. Besides this, in parasympathetic nerves both the pre- and postganglionic fibers are cholinergic. This adds to the difficulties in the determination of the blocking effects of cholinolytic substances at the parasympathetic ganglia. While it is relatively easy to study pure gangliolytics (such as hexonium**) as they do not exert a practical influence upon the effector cells innervated by the postganglionic fibers, it is much more difficult to examine the ganglionic actions of substances blocking both the M- and the N-cholinergic systems*** as in these instances the M-cholinergic effect tends to mask the action on the ganglia.

While studying the pharmacological properties of cholinolytic substances having both nicotinolytic as well as atropine-like properties, we became convinced that the methods so widely employed for the studies of the blocking effects upon the parasympathetic ganglia of these substances (arterial pressure, bowel in situ and others) are far from satisfactory. In these experiments the absence of a reaction to stimulation of the preganglionic fibers of the parasympathetic nerves in the presence of a response to acetylcholine is taken to prove a ganglionic cholinolytic block. However, the lack of a response to electric stimulation may also depend largely on the atropine-like properties of the preparation. In connection with this, it is interesting to evaluate the relative merits of methods used to investigate the effects of cholinolytic substances upon parasympathetic ganglia. We correlated experiments upon arterial pressure, the heart and intestine in situ. In this study we present the results obtained when we used atropine-like substances, specifically, the chlorhydrate ester of diphenylglycolic acid and diethylamino-ethanol (ester 22).

Experiments recording cat arterial pressure showed that small doses of ester 22 removed first the effects of vagal stimulation while it took much larger doses to remove the acetylcholine effects (Figure 1). This gives reason to believe that ester 22 has a powerful ganglion-blocking effect while having only weak atropine-like properties. However, the results of experiments with a cat heart in situ were somewhat different. The inotropic and chronotropic effects of acetylcholine (10-20 gamma/kg intravenously) and electric stimulation of the peripheral end of the cut vagus were abolished by the use of approximately the same doses. In many of the experiments the

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**Transliterated – probably hexamethonium.

***That is, substances possessing ganglion-blocking as well as atropine-like properties.

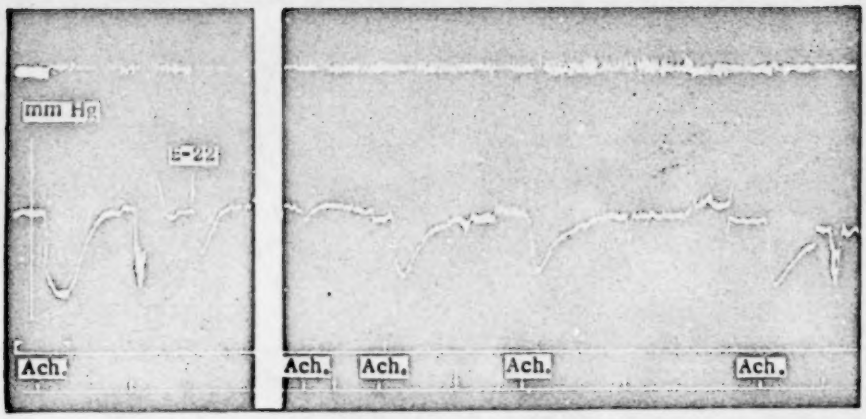

Fig. 1. Effect of ester 22 upon respiration and arterial pressure in a cat when the vagus nerve is stimulated with electric current and acetylcholine. Significance of curves (from above down): respiration, arterial pressure, time marker (1 second); stimulus marker: ↑) acetylcholine (1 gamma per 1 kg intravenously); ↓) vagus stimulation; E-22) ester 22 (0.2 mg/kg intravenously).

acetylcholine effects were abolished with somewhat smaller doses than were the electrical effects of stimulating the peripheral end of the cut vagus (Figure 2). Analogous data were obtained also in experiments done upon the bowel in situ. Bowel contractions produced by acetylcholine in most instances disappeared with smaller amounts of the preparation than were needed to abolish the contractions following the stimulation of the peripheral end of the severed vagus nerve.

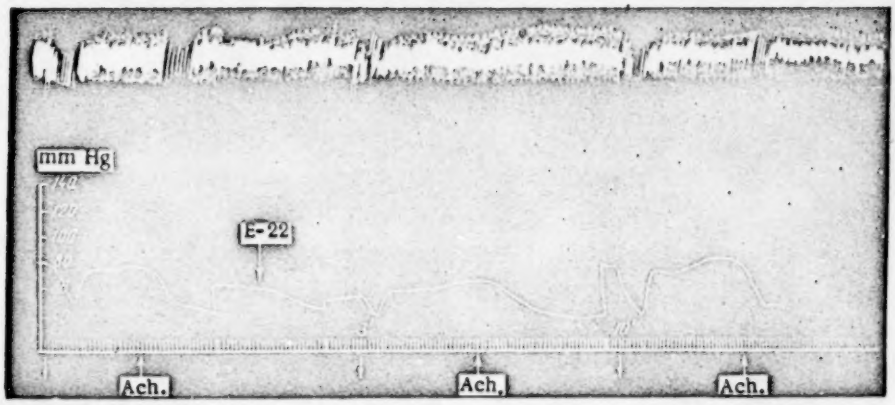

Fig. 2. Influence of ester 22 upon the arterial pressure and frequency and rhythm of cardiac contractions after vagus stimulation and acetylcholine action. Significance of curves (from above down): heart action in situ, arterial pressure, time marker (1 second); ↓)vagal stimulation; E-22) ester 22 (0.1 mg/kg intravenously); ↑) acetylcholine.

Thus, on the basis of cardiac and intestinal experiments in situ one might conclude that ester 22 has mainly atropine-like properties while, at the same time, one could interpret the arterial pressure experiments as in-

dicating that the preparation has mainly a ganglion-blocking action. This shows that the three most widely used methods in the determination of atropine-like and ganglion-blocking effects of substances fail to give an adequate concept of ester 22 actions.

In the experiments upon the heart in situ, simultaneously with the cardiac contractions, we recorded the arterial pressure. The results of our experiments showed that the cardiac and arterial pressure effects of electrical vagal stimulation disappeared with small dosages while the acetylcholine effects were abolished from the heart first while the arterial pressure required much larger doses (see Figure 2). These data indicate that the hypotensive effects evoked by electric stimulation of the vagus and acetylcholine are not equivalent and that removal of the vagal hypotensive effect during the response to acetylcholine is not evidence for the ganglion-blocking action of the given preparation. In this regard, the experiments on the heart and intestine are much more conclusive. But even here it is rather difficult to decide whether the removal of the vagal effect is due to ganglionic block by the given substance or whether it is due to its atropine-like properties.

To study the blocking action of cholinolytic substances on parasympathetic ganglia we chose the ganglia of the pelvic nerve. It turned out that our method of recording in situ the cat urinary bladder contractions was the method of choice for our investigation.

The studies were conducted as follows. By means of a catheter inserted into a dissected urethra the bladder was filled with warm Ringer solution and then, using a water manometer, it was connected with a Marey capsule. Bladder contractions were induced by stimulating the preganglionic parasympathetic fibers of the bladder branch of the pelvic nerve, using for this purpose either an induction current or intravenous injection of nicotine (0.02 mg/kg).

In these experiments it was established that atropine in doses of 5-10 mg/kg had almost no effect upon bladder contractions induced by intravenous nicotine or electric stimulation of the urinary branch of the pelvic nerve. Hexonium and nicotine (the first in doses of 1-2 mg/kg intravenously, the second in doses of 0.1-0.5 mg/kg) completely abolished urinary bladder contractions caused by electric stimulations of the pelvic nerve. Insofar as the bladder contractions caused by electric stimulation of the pelvic nerve or by nicotine remained stable in relation to atropine, this method presented many conveniences in the investigation of the action of cholinolytic substances having atropine-like qualities in their effects upon the parasympathetic ganglia of the pelvic nerve. Ester 22 in these experiments did not abolish bladder contractions even when administered in doses of 10 mg/kg (Figure 3). Thus, it is evident that the atropine-like preparation, ester 22, has almost no effect upon the parasympathetic ganglia of the pelvic nerve. When this preparation was used to study arterial pressure and the heart and intestinal actions in situ, it abolished the effects of electrical stimulation of the parasympathetic nerves even when used in doses of 0.1-0.8 mg/kg. Apparently, in these experiments the suppression of the effects of the electrical stimulation is the consequence of a block of the M-cholinergic system.

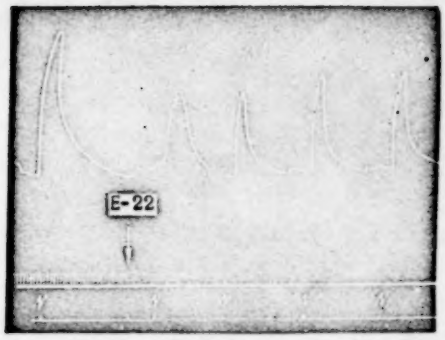

Fig. 3. Influence of ester 22 upon the parasympathetic ganglia of the pelvic nerve. Significance of curves (from above down): bladder contractions in situ; time marker (5 seconds); N) nicotine (0.02 mg/kg intravenously); E-22) ester 22 (10 mg/kg intravenously).

Our pharmacological analysis has demonstrated that the M-cholinergic system of the urinary bladder displays very little sensitivity to choline mimetic substances. Thus, pilocarpine, which is a selective stimulator of the M-cholinergic system [1], could produce only sporadic urinary bladder contractions when given intravenously in large doses such as 1-10 mg/kg. When repeated, pilocarpine as a rule failed to produce an effect. In favor of our hypothesis is the fact that bladder contractions caused by acetylcholine are prevented completely by intravenous use of nicotine (0.1-0.5 mg/kg) and by hexonium (1 mg/kg). Most likely, acetylcholine produces bladder contractions by stimulating the N-cholinergic system of the parasympathetic ganglia and not the M-cholinergic system of the muscle fibers. Otherwise nicotine, which acts selectively upon the ganglia, would not inhibit bladder contractions evoked by acetylcholine.

The lack of sensitivity displayed by the M-cholinergic system of the urinary smooth musculature to choline mimetic and cholinolytic substances makes the method we have described above quite useful for the evaluation of the blocking actions of cholinolytics upon parasympathetic ganglia.

The above discussion attests to the fact that there is no single method which, by itself, gives a complete picture of the ganglion-blocking actions of various substances. The most complete and trustworthy picture of the blocking actions of cholinolytic substances upon parasympathetic ganglia may be obtained by using all the known methods including experiments upon the urinary bladder.

SUMMARY

The most authentic data on the blocking effect of cholinolytics on the parasympathetic ganglia may be obtained only when different methods, including experiments on the urinary bladder, are employed.

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SEROLOGICAL ACTIVITY OF TISSUE LIPIDS IN MAN

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The immunological specificity of lipids has been studied little as yet. A number of works have been published [7, 8, 9] in which alcoholic extracts of different organs of various animals have been examined serologically. The authors have proved indirectly that lipids possess a species and organ specificity. Recently, some investigators from other countries have studied the active lipid components isolated from tumor tissue [10, 11, 12]. When rabbits were immunized with the mitochondrial fraction of rat lymphosarcoma, complement-fixing antibodies were formed which demonstrated a relatively high titer against the lipid components of this fraction. The antisera obtained did not react in the complement-fixation reaction (CFT) with lipid haptens from the tissues of rabbits, guinea pigs, mice, humans, or large cattle. The authors concluded from this [13] that lipids possess a species specificity. Ravich-Shcherbo and associates brought forth evidence in a series of papers of the antigenicity of lipids and proved the presence of a species immunological specificity in substances of a lipid nature [5]. The sera of rabbits, which had been immunized with an emulsion of dog cardiolipid in a physiological solution, gave a strong positive reaction in the CFT with dog lipids and a weaker reaction with lipids of other animals (bovine, rabbit) and humans.

The dearth of knowledge about the immunological specificity of lipids no doubt arises from the fact that a study of lipids is difficult because of their marked anticomplementary effects and their insolubility in a physiological solution – the usual medium for all serological reactions. Immunological results of a complete serological investigation of lipids cannot help being influenced by the application of organic solutions – alcohol, ether, acetone, benzene, etc. – in which the lipids are easily dissolved.

The purpose of the present work was a description of several new procedures, which facilitated a serological study of lipids, and a statement of the results of their immunological specificity.

METHODS AND RESULTS

The experimental lipids were extracted with chemically pure ether from normal and malignant tissue from the stomach and spleen of humans. The lipid fractions were dissolved in ether, warm alcohol, and acetone without a sediment, and in water they gave unstable emulsions. The presence of glycerin was demonstrated by a positive acrolein reaction. Serological analyses were done on the free lipids, which were extracted directly from the tissues in a Soxhlet's apparatus, and on the bound lipids, which were extracted with ether and then weakly hydrolyzed with 0.1 N acetic acid. The obtained ether extracts were dehydrated with heated sodium sulfate for removal of all water-soluble substances and were evaporated until dry in a water bath at 40°, after which the dry residues, brought to uniform weight, were dissolved in a specific volume of pure ether.

Portions of Whatman No. 1 chromatographic paper were weighed on a torsion balance (10-15-20 mg) and were saturated with equal quantities of the ether solution of lipids. The ether quickly evaporated, and the lipid-

treated paper was taken as the test antigen for the serological investigation. For serological properties of the paper-fixed lipids we used the quantitative CFT at 50% titer, developed in the Heidelberger laboratory and slightly modified by A.P. Konikov [3]. At the suggestion of V.S. Gostev we applied the "paper" azo-antigen of N.A. Shagynova [6] in the CFT at 50% titer. Squares of paper lipid-antigen were cut into small pieces, and the experimental serum, complement (always in excess), and physiological solution were added to them. The standard hemolytic system was used for the reverse titration of free complement not bound by a specific complex. The degree of hemolysis was determined by an electrophotocolorimeter. The number of free units of complement were calculated according to the formula of Crook:

$$\log X_2 = \log X_1 - n \log \frac{y}{100 - y}.$$

where X_2 is the volume of mixture capable of producing 50% hemolysis; X_1 is the volume of experimental suspension; $n = 0.2$ (constant value); y is the percent of hemolysis. The unit of complement was that amount which produced 50% hemolysis under the conditions of the experiment. In order to avoid protracted calculations, a direct graphic relationship was established between the electrophotocolorimetric readings and the number of free units of complement. The difference in the units of free complement in the control and experimental test tubes served as an indicator of the number of units of bound complement taking part in the formation of a specific compound.

In the investigation of the serological activity of human tissue lipids, serum from horse No. 343, which had been immunized with human tissues, was used as an antiserum. A natural lipid substance was taken as a control – butter, which could not react with "antihuman" serum because of a different species specificity. The results demonstrated a specificity: human tissue lipids, fixed on paper, bound complement with "antihuman" serum, while butter, impregnated on paper, did not react with this antiserum.

TABLE 1

CFT at 50% Titer With 15 mg "Paper" Lipid-Antigen (in units of complement)

Date of experiment	Lipid test antigen	Control antigen	Normal horse serum		Horse serum No. 343	
			units of complement			
			free	bound	free	bound
June 19-20, 1956	Saline extract of human spleen	24.2	24.2	0	13.55	10.65
	Butter	24.2	24.2	0	24.2	0
	Control: complement	28.3				
	Control: serum		28.5		24.2	
August 1-2, 1956	Saline extract of human stomach	5.53	5.15	0.38	0	5.53
	Butter	5.43	6.00	0	4.65	0.78
	Control: complement	10.6				
	Control: serum		9.85		9.85	

For further proof of the specificity of the reaction of human tissue lipids and antiserum, another control was set up, namely: the reaction between lipids and normal horse serum, which did not possess an antibody against human tissues. In this case the complement was not bound. The same results were obtained in other CFT with human lipid tissues and horse sera as well as rabbit sera. Hence, human tissue lipids reacted specifically with "antihuman" serum.

In view of the fact that milk lipids, such as the butter, could be completely different according to their serological properties, in further experiments we replaced the butter with lipids, extracted by exactly the same technique as in the first experiments, from stomach and spleen tissues of large cattle. "Antihuman" horse serum

No. 343 and normal horse serum were used as the antisera in these experiments. The findings, presented in Table 2, indicated that human lipid tissues reacted decisively with horse serum No. 343 against human tissue, specifically binding complement to it. But bovine tissue lipids did not react with this serum; neither human lipids nor bovine lipids reacted with normal horse serum not possessing a corresponding antibody.

TABLE 2

CFT at 50% Titer With "Paper" Lipid-Antigens of Humans and Large Cattle and Antihuman Horse Serum No. 343

Species source of lipids	Lipids from saline tissue extracts	Control antigen, units of complement	Normal horse serum		Horse serum No. 343	
			units of complement			
			free	bound	free	bound
Human	Malignant stomach	15.2	15.33	0	11.86	3.34
	Normal stomach	19.6	18.87	0.83	16.17	3.43
	Spleen	19.5	18.9	0.6	17.4	2.1
Large cattle	Normal stomach	10.4	10.28	0.12	10.0	0.4
	Spleen	11.24	12.0	0	11.10	0.14
Control	Complement	19.3				
	Serum		18.13		18.43	

The obtained data give rise to the assumption that there was a species specificity present in lipid fractions. For proof of such a possibility a number of cross-reactions were established with tissue lipids of different animals.

Precipitating sera used in the investigation were obtained from the Scientific Research Institute of Forensic Medicine of the Ministry of Public Health USSR. From Table 3 it is seen that lipids extracted from the stomach tissue of the cow reacted with precipitating anti-bovine serum and did not react with anti-dog serum. On the other hand, lipids from the stomach of the dog bound complement with anti-dog serum and did not react with serum against large cattle. From the results of December 28th and 29th, 1956, it is seen that in the system of tissue lipids of dogs + serum against large cattle there occurred a rather noticeable nonspecific binding of complement (7.62 units), but the specific binding of complement was considerably less (19.3 units).

TABLE 3

Cross-Reactions of CFT at 50% Titer With Tissue Lipids of Various Species Origins

Date of experiment	Species source of lipids	Control, unit of complement	Precipitating serum against tissue			
			dogs		large cattle	
			units of complement			
			free	bound	free	bound
December 26-27, 1956	Dogs	24.5	18.7	5.8	23.5	1.0
	Cow	25.2	23.7	1.5	19.3	5.4
	Control:					
	complement	25.2				
	serum		25.4		24.9	
December 28-29, 1956	Dogs	30.0	10.7	19.3	22.38	7.62
	Cow	30.0	30.0	0	13.68	16.32
	Control:					
	complement	30.0				
	serum		30.0		30.0	

Because of the strong anticomplementary action existing in our system of precipitating sera, we tested the serological properties of tissue lipids by the other quantitative serological reaction, the specific increase

of protein in the "paper" antigen, which was developed in our laboratory by V.S. Gostev. D.G. Grigorian studied the serological properties of desoxyribonucleoproteins in this reaction [2].

The preparation of the "paper" lipid-antigen was the same as in the setting up of the CFT at 50% titer. An accurately weighed square of chromatographic paper with lipids fixed on it was placed on the depressed surface of a concave slide (Maksimov chamber) and 0.3 ml of whole serum was added to it. The "paper" antigen and the treated serum were then incubated at room temperature in a humid chamber for one hour, after which the unstably-fixed proteins were washed off with three changes of a physiological solution during the course of a minute. After washing, the nitrogen content was determined three times by the Conway method. The experiment was always accompanied by standard controls of the reagents, the nitrogen content in the adsorbent – the pure chromatographic paper – and the nitrogen content in the adsorbent impregnated with the experimental lipids and the untreated sera. A little protein was detected in the pure adsorbent, which was probably due to a small amount of protein impurities in the paper. We did not succeed in demonstrating 20 mg lipid fractions by the Conway method. A specific increment of protein was tested in tissue lipids from the stomach and spleen of large cattle, dogs, swine, and man. For antisera we tried precipitating rabbit sera against the tissue of man, dogs, swine, and large cattle, horse antiserum No. 343 against human tissue, and normal horse serum.

We were able to compare quantitatively the protein increment in the lipid system – the homologous serum in the species relationship from the same lipid system with other sera, both normal and that obtained against other animal species.

TABLE 4

Cross-Reactions of Protein Increase in mg, Using Lipid "Paper" Antigens of Unlike Species and Sera

Species origin of tissue lipid	Nitrogen in lipid antigen (in mg)	Adsorbent + lipid + precipitating sera against tissue				Specific protein increase (in %)
		dogs	large cattle	swine	man (serum No. 343)	
Dog	0	0.2100	0.1400	0.0525		50.0
Large cattle	0.0	0.0262	0.1925	0.1137		69.3
Swine	0.00875	0.1400	0.0787	0.2625		87.5
Man	0		0.2887	0.3062	0.5250	71.4

In Table 4 some cross-reactions are presented of the protein increase from dog, swine, bovine and human lipids. From Table 4 it is seen that dog lipids in the complex with serum against dog tissue gave a specific protein increase of 50% in comparison with the nonspecific increase in the system of these same lipids and sera against tissue of man, large cattle, and swine. We observed an analogous picture with bovine, swine, and human lipids – a definite specific protein increase of 71.4%, 87.5%, and 69.3% in the presence of homologous serum in the species relationship.

The given reactions of a specific increase of protein completely corroborated the results obtained in the CFT at 50% titer: lipids fixed on paper gave a definite specific reaction in the presence of homologous serum in the species relationship. Evidently, the ether-soluble fraction of animal tissues is characteristic of the species specificity. Extraction of the tissue "lipids" with ether after acid hydrolysis of the tissue extracts must have led to decomposition of the lipoproteins. We were not able to detect nitrogen in the consumption of the 20 mg lipid fraction, and this indicated that there were no protein impurities in the fraction. However, we could not exclude the possibility that there might be nitrogen present in the experimental fractions in such negligible amounts that we could not determine it by the Conway method. Therefore, in speaking of lipids at the present time, we are referring to the fraction in which there are possibly infinitesimal protein impurities.

A more elaborate clarification and study of the immunological specificity of lipids is the objective of our further investigations, but this does not prevent at the present time a study of the serological properties of the paper-absorbed lipids and other substances insoluble in physiological solution, which are used in the CFT at 50% titer and in the protein-increase reaction.

The "paper" lipid antigen technique is highly sensitive and can serve as a quantitative determination. It permits a comparison of the serological activity of different antigens independent of their anticomplementary

action; in reactions of a specific increase of protein it can utilize sera having a high anticomplementary action; the prerequisites of the designated reactions are more standardized than in the usual classical CFT; an appraisal of results is more objective and possesses a numerical interpretation in units of bound complement or in milligrams of protein.

SUMMARY

A method of serological quantitative reaction was developed with lipid test-antigens fixed on paper, i.e., complement fixation test with 50% titer and reaction of specific addition of protein in "paper" antigen. The serological activity of various tissue lipids (free lipids) isolated directly from the tissues and combined (obtained from the saline extracts after preliminary hydrolysis) was demonstrated by these methods. Tissue lipids of man, dog, swine and large cattle react only with homologous antiserum.

This allows the suggestion that the tissue lipid fraction is species-specific.

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QUANTITATIVE STUDY OF IMMUNE HORSE SERUM ANTIBODY AGAINST CLOSTRIDIUM OEDEMATIENS BY MEANS OF PRECIPITATION ON PAPER OR PAPER-FIXED ANTIGEN

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Great significance is attached to the determination of antitoxin content in various immune sera. At the present time the basic method is a study of the neutralizing capacity of serum with respect to toxin, and the degree of neutralization is established in experiments with living animals. The antitoxin content of immune sera against one of the gas-gangrene agents, Clostridium oedematiens, is determined in a similar manner under industrial conditions.

The use of experiments with animals for antibody determination has a number of substantial disadvantages: it requires considerable time, a great number of livestock, and only gives an approximate answer. It is not customary to determine antitoxin content in sera by the flocculation technique, although references are sometimes encountered in the literature concerning the feasibility of flocculation methods in similar systems [4].

We have developed methods of quantitative determination of antibody content by means of precipitation on paper [2], or by using antigen fixed on paper [3]. We undertook a study of the possible application of these methods in the determination of antibody content against Cl. oedematiens for the purpose of replacing the serum titration in animals by more accurate and speedy biochemical techniques. We were especially interested in the problem of the quantitative dependence of the flocculation of Cl. oedematiens antitoxins with corresponding immune sera.

EXPERIMENTAL METHOD

For the investigation we used Cl. oedematiens antitoxins which were obtained from E.V. Vlasov in the Department of Wound Infections of the N.F. Gamaleia Institute of Experimental Microbiology. The antisera, which were recovered through the immunization of horses, contained 2000 antitoxin units and were kindly submitted to us by O.A. Komkov of the same department. In the electrophoretic study of these sera it was found that the γ-globulin content was about 40%.

The Cl. oedematiens antitoxins were concentrated and purified by A.P. Kuz'min by sedimentation with hydrochloric acid in the isoelectric zone after a preliminary application of sodium chloride. Further purification was accomplished by sedimentation of the antitoxins with acetone in the cold with a calculation of the tempera-

Fig. 1. Flocculation reaction on paper: A) point of application of antigen; B) point of application of antigen and immune serum; C) point of application of immune serum.

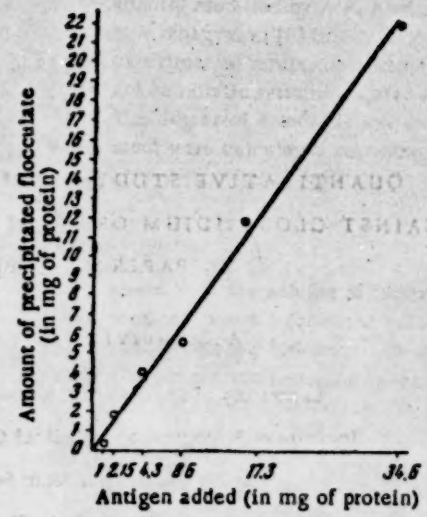

Fig. 2. Dependence between amount of antigen added and amount of precipitated flocculate.

ture, pH, and ion intensity [1]. The final result of the calculation amounted to 17 mg of protein in 1 ml.

The possibility of precipitation of a flocculate with the reacting antitoxins of Cl. oedematiens and the corresponding antisera was tested by the immunochemical method. A liberal sediment precipitated with the combination of equal volumes of antitoxin and antiserum. The relation between the amount of antigen added to the serum and the magnitude of precipitated flocculate was studied by the method of precipitation on paper [2]. These findings corresponded to the results obtained with the application of antiserum on paper discs with fixed antigen [3].

In the first series of experiments of the precipitation reaction on paper we studied the variation in the magnitude of precipitation with increasing doses of antigen added to constant volumes of antisera.

16.9 μl of immune serum was placed on several sections of chromatographic paper by means of an automatic micropipette. After the serum had dried, varying amounts of antigen were placed on the same spots. We inserted the sheet in the chromatographic chamber in such a manner that the border, where the antiserum had been applied, was immersed in a tray with veronal buffer, pH = 9.2. The buffer, flowing along the paper, carried off all the protein except the flocculate (Figure 1). The proteins from the section of paper containing the flocculate were extracted with 1 N NaOH for two hours at room temperature, the amount determined according to Lour [6], and the given quantity brought up to a volume of 1 ml.

EXPERIMENTAL RESULTS

Results of one of these experiments are presented in Figure 2. It shows that in the zone of excess antibody there existed a direct proportion between the amount of antigen introduced onto the paper and the magnitude of flocculate obtained. This proportion was maintained until all of the antibody separated out into the sediment, after which there began a diffusion of the precipitate in the excess antigen (Table 1).

In the system studied the relations between the amount of added antibody and the magnitude of sediment were entirely different from the well known system of horse serum albumin and antiserum [5]. In Figure 3 it is seen that the proportion between the magnitude of deposited sediment and the amount of added horse albumin

TABLE 1

Relation Between Amount of Antigen Added and Magnitude of Flocculate

Sample No.	Amount of antigen added in mg per ml of serum	Magnitude of flocculate	Amount of antigen*
1	8.6	6.1	–
2	17.3	10.1	–
3	34.6	20.4	18.4
4	69.2	14.9	–
5	148.4	13.6	–

*It was assumed that the precipitate contained 90% antibody [5].

was sharply changed according to the extent of increase of the amount of added antigen (Figure 3a). In contrast to this, the relation remained constant for a very broad zone of antigen concentration in the system of Cl. oedematiens antitoxin and immune serum (Figure 3b). Thus, the question arose about the relationship between the amount of antigen and antibody in the flocculate. Additional experiments were necessary to explain this.

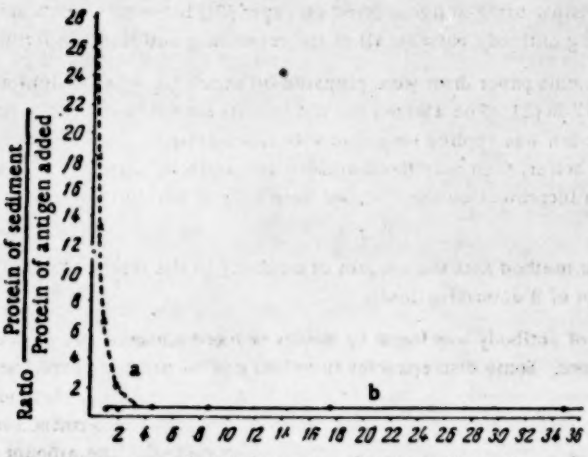

Fig. 3. Variation in the relationship between the amount of sediment and the amount of antigen added according to the degree of increased concentrations of antigen: a) system of horse albumin and immune rabbit serum; b) system of antitoxin and immune horse serum.

However, if one conditionally accepts that there is about 10% antigen contained in the flocculate, as it has been found for other systems [5], then the amount of antibody in 1 ml of the studied serum was equal to 18.4 mg.

In order to convince ourselves that we could judge the amount of antobody in the serum on the basis of our data, we tried a determination of antibody content with different dilutions of one of the immune sera at our disposal (Figure 4). It turned out that the amount of antibody was conversely proportional to the dilution of serum.

The antitoxin of Cl. oedematiens studied by us presented an intricate complex of six antigens [7]. In the study of the antigen composition of this antitoxin by the method of precipitation on agar, we observed the forma-

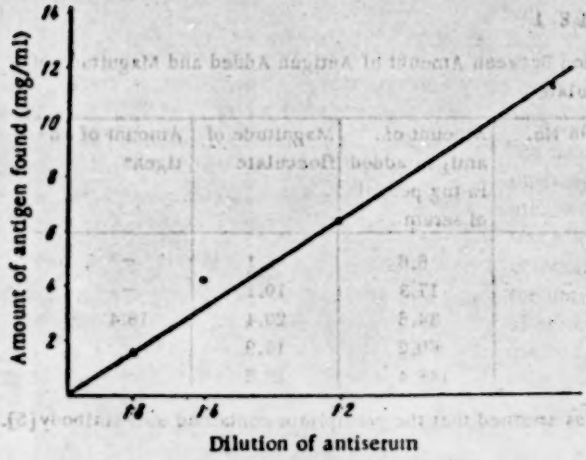

Fig. 4. Relation between amount of antibody found by described method and dilution of antiserum studied.

tion of five to seven zones of precipitation. It is possible that not all of the antibody, which was formed in the immunization of this complex mixture, precipitated in the reaction with the corresponding antigens. We applied a method of antibody determination using antigens fixed on paper [3], inasmuch as it allowed us to determine not only the amount of precipitating antibody but also all of the remaining antibody not forming a precipitate.

For this series of experiments paper discs were prepared on which Cl. oedematiens antigen was fixed chemically by an excess of alkyl halide [3]. The antigen did not lose its immunological activity with fixation in this manner. Hence, if immune serum was applied on a disc with fixed antigen, and all the unreacted protein was removed by a stream of veronal buffer, then only fixed antigen and antibody attached to it remained on the disc. Having determined the protein increment on the disc, we were able to determine the amount of antibody in the experimental serum.

It was established by this method that the amount of antibody in the serum against Cl. oedematiens consisted of 17.53 mg/ml (an average of 9 determinations).

Thus, the same amount of antibody was found by means of fixed antigens as in the determination by the method of precipitation on paper. Some discrepancies in values can be partially explained by different methods. In addition, it is possible to determine only the amount of antibody protein by the method of fixed antigen, but by the method of precipitation on paper the entire amount of protein can be determined in the sediment, where an accurate antigen-antibody relationship is unknown in the system studied. The amount of antigen in the sediment can be somewhat higher than the 10% which we obtained.

Both methods conform in their results in respect to an absence of an appreciable amount of nonreacting antibody in the studied serum. Thus, serum antibody against Cl. oedematiens by both techniques represents the actual amount of antibody. Application of chemical methods is considerably more accurate than serum titration in animals and can be accomplished in a briefer period of time.

However, one must take into account that because of the complexity of the antigen formation of Cl. oedematiens antitoxin and the presence in the serum of a number of antibodies, the over-all determination of their general amount did not allow us always to obtain a detailed characterization of the immune serum. For future investigations of a similar nature individual fractions separated from the antitoxin will be desirable to use as antigen.

SUMMARY

Reaction of flocculation between the antitoxin of Cl. oedematiens and the corresponding immune horse serum was studied with the aid of precipitation on paper. When toxin and immune serum were placed on the same

place on the paper, a flocculate appears, which unlike all the rest of the proteins is not washed off the paper by the veronal buffer. The flocculate is precipitated at the zone of considerable surplus of antitoxin. The quantity of precipitate is increased in proportion to the quantity of the added antigen up to the time when the whole quantity of the antigen is precipitated. Then the precipitate begins to dissolve in the surplus of the antigen. Data which were obtained by the method of precipitation on paper correspond to the results of determination of the quantity of the antibodies in the same serum with the aid of the antigen fixed on paper through diazoconnection and the residual haloidalkylate. This points to the possibility of replacement of the biological method of determination of the antigens of Cl. oedematiens by the immunochemical method.

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ONCOLOGY

THE ANTIGENETIC PROPERTIES OF "HUMAN LEUKEMIC FACTOR" CULTIVATED ON THE CHORIOALLANTOIC MEMBRANE OF THE CHICK EMBRYO

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In the study of the properties and nature of human "leukemic factor" [1] the possibility of its cultivation on the membranes of the developing chick embryo is particularly important.

In order to prove that this agent may be actively cultivated on the membranes of the chick embryo three main methods are employed: testing the specific biological activity of the culture material in animals, study of the culture material with the electron microscope and determination of its specific antigenic properties in immunological reactions. In this report we give the results of a study of the specific antigenic properties of the leukemic factor.*

As we know from the work of L.A. Zil'ber, V.A. Parnes [3] and others, a specific leukemic antigen is present in the bodies of leukemia patients.

The main purpose of our work was to find out: does the allantoic fluid of an embryo in which blood from leukemia patients has been inoculated contain a factor analogous to the specific leukemic antigen contained in the blood of patients with leukemia?

We found no evidence in the literature of the possibility of cultivation of material obtained from leukemia patients in the developing chick embryo.

EXPERIMENTAL METHOD

Blood from patients with acute leukemia was cultivated on the chorioallantoic membrane of seven-day chick embryos. Inoculations were made once every five days using cortisone.

For detection of the leukemic factor and determination of its specific antigenic properties we used one of the most sensitive immunological reactions – L.A. Zil'ber's active anaphylaxis and desensitization reaction. The method of performing this reaction did not differ in principle from that used by L.A. Zil'ber and his co-workers [2, 3 and others] for demonstrating the specific antigens of malignant tumors (see Tables 1 and 2).

As antigens we used blood of leukemia patients, blood of healthy persons, allantoic fluid inoculated with

*The results of an investigation into the leukemia-producing activity of the cultivated agent together with a description of the method of its cultivation on the membranes of the chick embryo will appear in later reports.

blood* or marrow filtrate of leukemia patients; or allantoic fluid inoculated with blood of healthy persons.** Altogether 90 guinea pigs (weighing 250-300 g) were sensitized. The tables show the results of experiments on 70 guinea pigs; the remaining animals died or were removed from the experiment for various reasons. The protein content (by Kjeldahl's method) of the allantoic fluid inoculated with leukemic blood was 2.88 mg/ml; that of allantoic fluid inoculated with normal blood was 2.66 mg/ml.

EXPERIMENTAL RESULTS

The object of the first series of experiments was to find out if there were any specific antigenic differences between "leukemic" and "normal" allantoic fluid.

Guinea pigs which had been sensitized to "leukemic" allantoic fluid and desensitized to "normal" allantoic fluid were tested for the presence of specific sensitization by giving them an assaulting injection of "leukemic" allantoic fluid. Out of seven guinea pigs sensitized with a dose of 1.5 ml, a clear reaction was observed in five; in one animal the reaction was indefinite and in one it was not observed at all (Table 1, Experiment 8).

Of seven guinea pigs sensitized with a dose of 1 ml, two showed a definite reaction, three an indefinite reaction and two no reaction (Table 1, Experiment 7). The best results were obtained by sensitization of guinea pigs with 2 ml of allantoic fluid inoculated with marrow filtrate from a leukemia patient.

After desensitization to allantoic fluid inoculated with filtrate of normal marrow tissue, an assaulting injection of the antigen used for sensitization caused a clear anaphylactic reaction (Table 1, Experiment 10). These experiments revealed the decisive importance of the sensitizing dose and the time of sensitization: with large doses and a longer period of sensitization the anaphylactic reaction was more pronounced. This work also showed that it was better to use fresh than preserved antigen for the assaulting injection.

The results of these experiments provide evidence of the presence of a specific factor in "leukemic" allantoic fluid which is absent from "normal" allantoic fluid.

It was necessary to prove that this is the "leukemic" factor and not some chance contaminating antigen. For this purpose the following series of experiments was performed.

Guinea pigs were sensitized with fresh whole blood from patients with acute leukemia. Desensitization was carried out with healthy human blood or with allantoic fluid in which healthy human blood had been inoculated.

An assaulting injection of allantoic fluid inoculated with blood from a patient with leukemia caused a definite anaphylactic reaction in all 16 guinea pigs, which was not observed in control animals (Table 2, Experiments 2, 4, 5).

In this experiment attention is drawn to the absence of reaction in guinea pigs sensitized to leukemic blood on the injection of allantoic fluid inoculated with healthy human blood.

Thus, from the findings given it may be concluded that "leukemic" allantoic fluid contains a specific leukemic factor which is absent from "normal" allantoic fluid. As an additional control we made a special experiment in which guinea pigs sensitized with healthy human blood showed no anaphylactic reaction in response to injection of "normal" allantoic fluid nor to an assaulting injection of allantoic fluid inoculated with blood or medullary filtrate from a patient with leukemia.

The results of this experiment, as of Experiments 5 (Table 2) and 9 (Table 3) indicate that the components of normal blood cannot be cultivated on the membranes of the developing chick embryo.

Having discovered that in animals sensitized with blood of leukemia patients no reaction is observed to "normal" allantoic fluid, we performed the converse experiment. Guinea pigs were sensitized with "leukemic" allantoic fluid, and received as an assaulting injection blood from patients with leukemia or healthy human blood (Table 3).

This experiment gave clear results: while reaction to injection of healthy human blood into the guinea pigs was completely absent, a definite anaphylactic reaction was observed to injection of blood of the patient with leukemia.

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*In the future this will be referred to briefly as "leukemic" allantoic fluid.

**In the future – "normal" allantoic fluid.

TABLE 1

Experiment with Allantoic Fluid

Expt. No.	Sensitization			Desensitization								Assaulting injection		
	animal No.	antigen	dose (ml)	antigen	dose (ml) subcutaneously	reaction	dose (ml) intravenously	reaction	dose (ml) intravenously	reaction	dose (ml) intravenously	reaction	antigen	dose (ml)	reaction
8	41	"Leukemic" allantoic fluid (10th and 11th cultures)	1.5	"Normal" allantoic fluid (11th, 12th and 13th cultures)	1.0	—	0.3	++	0.6	—	1.5	—	"Leukemic" allantoic fluid (10th and 11th cultures)	1.5	—
	43		1.5		1.0	—	0.25	+++	1.0	++	1.5	—		1.5	+
	12		1.5		1.0	—	0.2	+	0.5	—	1.5	—		1.5	±
	44		1.5		1.0	—	0.2	+	0.5	—	1.5	—		1.5	+
	14		1.5		1.0	—	0.2	+	1.0	—	1.5	—	Ditto	1.5	+
	16		1.5		1.0	—	0.5	+++	1.0	—			19th culture with	1.0	+
	17		1.5		1.0	—	0.5	++	1.0	—	2.0	—	fresh material	1.0	+
		Control	—		—	—	—	—	—	—	—	—		1.5	—
7	1	"Leukemic" allantoic fluid (10th and 11th cultures)	1.0	"Normal" allantoic fluid (11th, 12th and 13th cultures)			0.1	+++	0.2	—	0.6	±	"Leukemic" allantoic fluid (10th and 11th cultures)	0.4	—
	2		1.0				0.05	++	0.25	+	0.7	—		0.7	±
	6		1.0				0.05	++	0.3	+	1.0	±		0.6	±
	7		1.0				0.05	+	0.3	—	1.0	—		1.0	—
	8		1.0				0.1	++	0.2	—	1.0	±		0.5	±
	9		1.0				0.05	++	0.2	—	1.0	—		1.0	+
	10		1.0				0.1	++	0.4	++	1.5	—		1.0	+
		Control	—				—	—	—	—	—	—		1.5	—
10	51	Allantoic fluid inoculated with medullary tissue extract from a patient with leukemia (11th and 12th cultures)	2.0	Allantoic fluid inoculated with normal medullary tissue extract (5th and 6th cultures)	2.0	—	0.3	—	1.0	+	2.0	—	Allantoic fluid inoculated with medullary tissue extract from a patient with leukemia (11th and 12th cultures)	1.5	+±
	52		2.0		2.0	—	0.2	—	1.5	+	2.0	—		1.0	+±
	53		2.0		2.0	—	0.3	—	2.0	+	2.0	—		2.0	++
	54		2.0		2.0	—	0.3	—	2.0	+	2.0	—		1.5	+
	55		2.0		2.0	—	0.3	—	1.5	+	2.0	—		1.0	+
	60		2.0		2.0	—	0.6	—	2.0	—	—	—		1.0	±
		Control	—		—	—	—	—	—	—	—	—		2.0	—

TABLE 2

Experiment with Blood from Patients with Leukemia

Expt. No.	Sensitization			Desensitization								Assaulting injection		
	animal No.	antigen	dose (ml)	antigen	dose (ml) subcutaneously	reaction	dose (ml) intravenously	reaction	dose (ml) intravenously	reaction	dose (ml) intravenously	reaction	antigen	dose (ml)	reaction
2	4	Blood of patient with acute leukemia. Group O(1)	0.25	Healthy human blood. Group O(1)	0.5	—	0.1	+	0.25	—	0.25	—	"Leukemic" allantoic fluid 6th culture of Group O(1) blood	0.9	+
	5		0.25		0.5	—	0.2	++	0.25	+	0.25	—		1.0	+
	9		0.25		0.5	—	0.1	+	0.25	—	0.25	—		1.5	+
		Control	—		—	—	—	—	—	—	0.25	—		1.5	—
4	7	Blood of patient with leukemia. Group O(1)	0.25	Healthy human blood. Group O(1)	0.25	—	0.2	++	0.5	—			"Leukemic" allantoic fluid 9th culture of Group O(1) blood	0.6	++
	8		0.25		0.25	—	0.2	++	0.5	—				0.5	+
	10		0.25		0.25	—	0.2	+	0.5	—				1.0	+
	16		0.25		0.25	—	—	—	—	—				0.6	+
	17		0.25		0.25	—	—	—	—	—				0.9	+
	18		0.25		0.25	—	—	—	—	—				1.25	+
		Control	—		—	—	—	—	—	—				1.5	—
5	1	Blood of patient with leukemia. Group O(1)	0.25	"Normal" allantoic fluid (9th culture) with healthy human Group O(1) blood	0.9	—							"Leukemic" allantoic fluid (9th culture of Group O(1) blood	0.9	+
	2		0.25		1.25	—								1.0	++
	3		0.25		1.25	—								1.0	+
	4		0.25		1.25	—								1.0	+
	5		0.25		1.5	—								1.5	+
	12		0.25		2.0	—								1.0	+
	13		0.25		2.0	—								1.5	+
		Control	—		1.5	—								1.5	—

TABLE 3

Experiment No. 9 With Allantoic Fluid

Sensitization			Assaulting injection		
guinea pig No.	antigen	dose (in ml)	antigen	dose (in ml)	reaction
31	"Leukemic" allantoic fluid	2.0	Blood of a patient with leukemia of Group O(1).	0.9	+
32		2.0		0.8	±
33		2.0		0.9	+
34	10th, 11th and 12th cultures	2.0		1.6	++
35		2.0		1.6	++
	Control	–		1.8	–
36	"Leukemic" allantoic fluid	2.0	Healthy human blood of Group AB (IV)	1.4	–
37		2.0		1.0	–
38		2.0		1.8	–
39	10th, 11th and 12th cultures	2.0		1.4	–
40		2.0	Whole blood of a Group A (II) donor	1.8	–

The results of this experiment thus also indicate the presence of a specific leukemia factor in allantoic fluid inoculated with blood from a patient with leukemia.

The above results give grounds for concluding that allantoic fluid inoculated with blood from patients with leukemia contains a specific leukemia factor. The fact that this factor can be cultivated for a considerable time (up to 19 passages) on the membranes of developing chick embryos testifies to its virus-like properties.*

SUMMARY

It was demonstrated with the aid of anaphylactic reaction with desensitization that the specific leukemic factor is present in the allantoic fluid of chick's embryos into which the blood of leukemic patients was injected. This factor is absent in the "normal" allantoic fluid. Human "leukemic factor" may be cultivated for a long time (up to 19 passages) on the chorion-allantoic membrane of developing chick's embryos.

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**In Russian.

AN EXPERIMENTAL STUDY OF THE ROLE OF ANTIBODIES IN IMMUNITY TO TUMORS

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Despite the large volume of work devoted to a study of the role of antibodies in the mechanism of protection against tumors, this problem remains unsolved. Of the greatest importance in this connection are investigations in which the role of antibodies is examined by their direct action on the malignant cell, i.e., by their effect on the vital functions of tumor tissue.

It has been shown in numerous reports [4, 5, 10, 15, 17, 20] that in the serum of animals immunized with heterologous tumor tissue antibodies appear which inactivate tumor cells in vitro and inhibit growth of this tumor in vivo. The antineoplastic action of the sera of animals immunized with heterologous tumor tissue is not, however, sufficiently direct proof of the role of antibodies in defense against tumors, for according to the findings of Bogomolets [1], Kavetskii and Fediushin [3], Vinogradova [2] and others, normal animal sera also exhibit a cytotoxic action on tumors of animals of another species.

This action is intensified not only by immunization of the donors with the corresponding tumor tissue but also by administration to them of extracts of spleen, thymus and other normal organs rich in reticuloendothelial tissue.

Of rather greater interest are investigations directed to the discovery of protective antibodies in animals susceptible to a particular tumor. Reports of these are few in number and contradictory in their conclusions [6, 7, 9, 14, 18].

We may dwell in some detail on certain of these investigations, made in recent years. Falls [11] performed experiments with Gardner lymphosarcoma, arising in mice of the C_3H strain and inoculated into mice of the CBA strain. Irradiation of this strain of mice with x-rays caused absorption of the tumor and subsequent immunity to further transplantation. On pairing immune mice parabiotically with nonimmune mice of the same strain (CBA), the latter also became immune to transplantation of the lymphosarcoma. Schreck and Preston [19] reported that transplantation and subsequent absorption of Bagg lymphosarcoma in rats of the Sprege-Daul'* strain is accompanied by the appearance in the serum of these animals of antibodies which inactivate the lymphosarcoma cells in vitro and cause severe cytological changes in the tumor cells; the sera have no action on cells of other tumors nor on normal rat tissues. Normal rat serum does not cause cytological changes and does not inactivate lymphosarcoma cells.

Gorer and Amos [12, 13] immunized mice of strains A, BALB/C and C_3H with tumor cells from mice of strain $C_{57}BL$ suffering from EL_4 leukemia. Injection of immune sera into mice of all the strains mentioned produced in them complete or partial inhibition of tumor growth after subsequent implantation of leukemic cells. Similar properties were acquired by the serum of BALB/C mice immunized with ascitic sarcoma BP_8: injection

*Transliterated from Russian.

of this serum into mice of strain C_3H, susceptible to sarcoma, was accompanied by inhibition of growth of the tumor in the mice. In contrast to the above experiment, Todd and Kidd [21] could detect no depressing action on tumor cells by sera of mice with a developing lymphoma or after absorption of this tumor. Negative results were obtained also in experiments by Miller [16]. It may be thought that the cause of failure was an insufficient intensity of immunization of the animals, with consequent feeble development of the antineoplastic properties of the test sera.

The purpose of this investigation was to ascertain the possibility of passive transmission of immunity against tumors to susceptible animals from immunized animals of the same species.

EXPERIMENTAL METHOD

The investigation was carried out on Brown-Pearce rabbit carcinoma. Rabbits of an impure strain, weighing 3-3.5 kg, were inoculated with a suspension of Brown-Pearce tumor intradermally. From five to seven days later at the site of injection intradermal tumors appeared and, in the majority of cases were reabsorbed. Afterwards the rabbits were reinjected 10-12 times with a suspension of the carcinoma. The intervals between the injections were 21-30 days. In this way the rabbits became highly resistant to inoculation with even high doses of tumor tissue. Blood was taken from the rabbits before the beginning of the vaccinations and on the 7th, 9th, 14th and 20th days after its conclusion. In all the experiments fresh serum was used, which had been stored in a refrigerator for not more than five days.

In the first series of experiments a fresh rabbit was injected intramuscularly with 10 ml of serum from an immune rabbit. After 24 hours this rabbit was inoculated in the muscle of the same or another limb with 1 ml of tumor suspension in a dilution of 1:5-1:10. In some experimental animals the serum was injected once only, in others up to ten times from the time of inoculation of the tumor material. The volume of serum injected varied from 10 to 60 ml per rabbit. As controls healthy rabbits were used, not subjected to any form of interference, or injected with normal rabbit serum only. The control rabbits were inoculated at the same time as the experimental rabbits. The animals were kept under observation for 1-1½ months, after which they were killed and examined post mortem.

EXPERIMENTAL RESULTS

The results obtained are shown in Table 1.

Altogether 17 immune sera were tested. A protective action was shown by four sera, three of which completely inhibited tumor growth and one partially. This was shown by the fact that the weight of the tumor in the experimental rabbit was 1.6 g while the weight of the tumor in the control animals was 15 and 20.5 g. It must

TABLE 1

Action of Immune Serum in Vivo

Serum	Number of rabbits in experiment	Number of immune rabbits	Number of rabbits with partial inhibition of tumor growth
Immune	51	5	1
Normal	10	0	0
Not injected	10	0	0

be pointed out that the tumor in the experimental rabbit resembled a continuous necrotic mass. The protective properties of each serum were tested in several experiments. Identical results were obtained. The effect of the serum depended on the time it was taken. For example, serum No. 188, obtained from blood taken on the 16th and 27th day after vaccination, completely inhibited growth of the tumor in fresh rabbits; the same serum No. 188 obtained on the 47th and 60th days after the conclusion of vaccination had no protective action. The volume of serum injected did not affect the results of the experiment. A negative action on the protective proper-

ties of the serum was shown by excessive hyperimmunization and frequent taking of blood from a rabbit. For example, serum No. 375 gave good results after the fourth and sixth vaccinations but had no action after the tenth vaccination.

Besides intramuscular injection of serum and tumor suspension, in three experiments we injected the serum and suspension intraperitoneally, using the method described above. The tumor suspension was injected in a dilution of 1:5 and a volume of 1 ml. In two experiments no difference was observed between the course of the tumor process in the animals of the experimental and control groups, and in one experiment the immune serum caused partial inhibition of tumor growth: spread of the tumor in the experimental animals was considerably more limited than in control animals, and the average weight of the tumors in the experimental animals was correspondingly 26 times less than in the controls injected with normal serum.

The results obtained are evidence of the possibility of passive transmission of immunity to Brown-Pearce tumors by means of antibodies. However, the fact that out of 17 sera obtained from highly immune rabbits, only four showed any protective properties, suggests that the antibody concentration of the sera is low. In some cases we were unable to detect such a low concentration of antibodies because in order to test the immunity very large doses of tumor material were injected (suspension in dilutions of 1:5-1:10). We used large doses because we were working with rabbits obtained from the market whose sensitivity to Brown-Pearce carcinoma varies widely. If we had used smaller doses of the suspension, tumors would not have developed in all the control animals, which would have made it impossible to evaluate the results obtained. In this case, in order to obtain statistically significant data it would have been necessary to include in the experiment a very large number of rabbits, which we could not have done. In a second series of experiments we produced transmission of immunity by means of transplantation of the spleen of immune rabbits into fresh animals.

The spleen of an immune rabbit was finely shredded with scissors into small pieces and transplanted intraperitoneally into two fresh rabbits in the region of the greater omentum. After 24 hours an intraperitoneal injection of 1 ml of tumor suspension diluted 1:5 was given to these rabbits and also to controls into which normal rabbit spleen had been transplanted. The rabbits were kept under observation for 1-1½ months, after which they were killed and examined post mortem. The results of these experiments are shown in Table 2.

TABLE 2

Transplantation of the Spleen of Immune Rabbits into Animals not Exposed to Any Form of Treatment

Material transplanted	Number of rabbits in experiment	Number of rabbits with complete inhibition of tumor growth	Number of rabbits with partial inhibition
Spleen of immune rabbits	17	3	2
Spleen of normal rabbits	18	0	0

Altogether nine spleens from immune and nine from normal rabbits were transplanted. Two immune spleens produced complete inhibition of tumor growth, and one partial inhibition, as shown by the fact that tumor growth in the experimental rabbits was considerably less intensive and widespread than in controls. The experiments which were carried out showed the possibility of transmission of immunity by means of transplantation into fresh rabbits of the spleen of resistant animals. However, in this case, the results obtained were feebly expressed, partly on account of the fact that in order to test the immunity we had to use very large doses of the tumor suspension.

In the third series of experiments we attempted to produce immunity by transplanting the lymphatic glands of immune animals into fresh rabbits. Rabbits resistant to Brown-Pearce tumors were immunized intradermally

in the posterior surface of the shank of the hind limbs. On the ninth day after immunization their popliteal lymphatic glands were removed. After being cut up with scissors they were transplanted into the peritoneal cavity of two fresh rabbits. Into control rabbits were transplanted lymphatic glands from normal animals. After 24 hours the experimental and control animals were injected intraperitoneally with tumor suspension. During the month after inoculation the control animals died. At necropsy multiple tumor nodules were found in all the internal organs, together with ascites. The weight of the tumors was about 200-220 g. The experimental rabbits were killed 1½ months after inoculation.

At necropsy of one rabbit a uniformly necrotic tumor nodule weighing 1.5 g was discovered in the parietal peritoneum at the site of injection. In a second rabbit solitary tumor nodules were found in the mesentery, weighing 10 g. In this experiment an obvious difference was found in the intensity of tumor growth in the experimental and control animals, but the experiment must be repeated using a larger number of animals.

The spleen and lymphatic glands are organs which produce antibodies, and transmission of immunity is effected in this case by means of antibodies. It must be pointed out that it is not only these antibodies which are found in the spleen and lymphatic glands of immune donors which play an important part, but also antibodies which continue to be produced by the transplanted tissues in the body of the recipient. Thus, the transmission of immunity from rabbits resistant to Brown-Pearce carcinoma to normal animals may be effected by means of antibodies present in the serum, spleen and lymphatic glands. So that the question of the nature of these antibodies – true tumor antibodies or isoantibodies – can be answered, the whole of this work must be reproduced in pure-bred animals in which a tumor has arisen and is kept going by implantation in other animals. This would allow the doses of tumor material in testing immunity to be reduced, and also would shed light on the nature of the antibodies formed.

SUMMARY

This work is devoted to the study of the role of the antibodies in induction of immunity to Brown-Pearce carcinoma. Serum or splenic tissue and lymph node tissue, obtained from rabbits immune to the tumor was introduced to rabbits. The subsequent inoculation of the tumor tissue to experimental animals was connected in a number of cases with complete or partial inhibition of the tumor growth. These data demonstrate the significance of the antibodies in the mechanism of antitumor protection of the organism.

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METASTASIS FORMATION BY BROWN-PEARCE TUMOR IMPLANTED IN THE BRAIN

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In previous experiments in which tumors were implanted into certain animal organs (testis, metaphysis of the femur) we noticed that the tendency to preferential involvement of particular organs shown by the tumor metastases varied in relation to the site of implantation. Similar findings were obtained by other workers [8, 9 and others]. The susceptibility of some organs and the resistance of others, related to the site of implantation and to the subsequent growth of the tumor, led us to the hypothesis that the differential distribution of metastases among the organs is determined not only by neurotrophic disturbances arising by reflex pathways in the affected organs, but also by protective and adaptive processes arising at the same time and in the same way in unaffected organs [11, 10, 12].

The aim of our work was to investigate metastasis formation in relation to implantation in various areas of the brain.

In human clinical medicine we find primary malignant tumors of the brain of varying structure and localization, and also secondary tumors of the brain – metastases from tumors of certain internal organs.

In the literature on experimental oncology some writers include the brain among tissues showing high resistance to tumor development [13], while others attribute low resistance to it [1, 4].

Pearce and Brown, while studying the rabbit tumor which they described, observed that on implantation into the brain the tumor develops without formation of metastases.

We implanted a Brown-Pearce strain of tumor in some rabbits in the tissue of the superficial layer of the brain, and in others in the lateral ventricle. Injections of 0.3 and 0.5 ml respectively of a 25% suspension of finely shredded tumor in physiological saline were made into the brain under ether anesthesia through a burr-hole in the skull.

The implantation in the brain tissue was carried out at the junction of the anterior and middle thirds of the left hemisphere, and the tumor suspension was injected into the upper layer of tissue at the same level, which was achieved by introducing the needle with a mark on it always to the same depth.

For implantation in the lateral ventricle the tumor suspension was injected through a needle in the anterior horn of the lateral ventricle.

Altogether 119 animals were included in the experiment.

In a preliminary series of experiments the tumor was implanted in ten rabbits in brain tissue and in ten

rabbits in the left lateral ventricle. At the same time in three control rabbits the same tumor was implanted in the testis.

In all the control animals, which died on the 32nd-38th day, tumors had developed in the testis, together with numerous metastases disseminated throughout the internal organs.

Of the ten rabbits in which the tumor was implanted in the tissue of the left cerebral hemisphere, in seven a tumor the size of a pea to a pine kernel developed. Of these animals, two rabbits died on the 42nd-50th day and the rest were killed after 60 days. In no case were metastases present.

Of the ten rabbits with implantation in the left lateral ventricle of the brain, tumors developed at the site of implantation – in the cavity of the anterior horn – in eight animals. Of these rabbits three died on the 40th day, five on the 50th-53rd days and the rest were killed on the 60th day. In six rabbits a tumor had developed in one ventricle, and in two rabbits it had extended also into the second lateral ventricle. In no case were metastases found in the internal organs. In one rabbit with a tumor in both lateral ventricles, paralysis of both hind limbs was observed during the last three days of life.

In order to analyze the results obtained, and in particular to elucidate what was responsible for the absence of metastases after implantation of the tumor in the brain tissue or the lateral ventricle, new series of experiments were called for.

In these experiments, following the example of the previous investigation, an additional stimulus was applied in the form of a single injection into the left testis of 0.5 ml of a 1% solution of formalin five days after implantation of the tumor in the tissue or lateral ventricle of the brain. Two series of experiments on 42 animals were set up. The same number of rabbits constituted two control series: in these animals implantation of the tumor was made in the tissue of the brain or in the lateral ventricle without the additional feature. The rabbits in which implantation of the tumor was made into the left testis (12 animals) served as a control of the activity of the tumor suspension used in the two experiments. All the animals were killed 35 days after implantation. The results of the experiments are shown in the table.

EXPERIMENTAL RESULTS

In the experimental groups of animals with both forms of implantation and with the additional stimulus, tumor metastases were found in certain internal organs in 13 rabbits. In the corresponding control groups (i.e., without the additional stimulus) no metastases were found in the internal organs.

The lateral ventricles on transverse section of the brain of a rabbit (No. 1123). A large tumor is seen in the left lateral ventricle extending into the right ventricle.

With implantation of the tumor in the brain tissue of the left hemisphere, the primary tumor nodules varied in size from a grain of buckwheat to a pine kernel. There was no significant difference in the dimensions of the primary nodules in the experimental and control groups. In only one of the experimental animals had the tumor spread in the upper layer of brain tissue of almost the whole anterior third of the hemisphere.

With implantation in brain tissue metastasis formation took place only in the presence of a primary focus. In five rabbits metastases were situated in the liver – from one to three nodules in size from a millet seed to a pea. In one of these cases (two pea-size nodules) the kidney was affected in addition to the liver (in one kidney – three nodules the size of a millet seed in the cortical layer).

With development of the tumor in the lateral ventricle, tumor growth in the control animals was restricted to the cavity of one ventricle. In the group with an additional stimulus, in seven rabbits the tumors extended into the cavities of both ventricles (see figure). In six of these animals there were metastases also in internal organs. Metastasis formation was observed always only in the presence of a primary tumor focus, when there were metastases in the liver in seven rabbits, in three of them with simultaneous involvement of the kidneys. In one rabbit the kidneys alone were

affected. Metastases in the liver appeared as one to four nodules in size from a millet seed to a pea, in the kidneys – one to three nodules from a millet seed to a grain of buckwheat, situated in the cortical layer. In three animals the kidney lesions were bilateral.

In one animal with metastases a bilateral paralysis of the hind limbs was observed at the end of the experiment. Metastases in the spine were not found.

The results which we obtained in our experiments show (see table) that implantation of a tumor of the Brown-Pearce strain into the brain tissue or the lateral ventricle of the brain does not lead to metastasis formation in the internal organs. In not one of the 42 animals in which this implantation was performed were metastases found despite the growth of the tumor. It might be thought that by virtue of some reason the cells of the implanted tumor, despite growth of the tumor in the brain tissue, do not infiltrate the vessels and do not enter the general circulation.

Metastasis Formation by Brown-Pearce Tumors Implanted in the Brain With the Application of an Additional Stimulus

Nature of the experiment	Number of rabbits in the group	Number of rabbits		Number of organs with metastases
		with a tumor at the site of implantation	with metastases in internal organs	
First group of animals				
Implantation of tumor in brain tissue; 5 days later injection into the testis of 0.5 ml of 1% formalin solution.	20	18	5	6
Second group of animals				
Implantation of tumor into brain tissue (control).	20	16	0	0
Third group of animals				
Implantation of tumor in the lateral ventricle of the brain; 5 days later injection into the testis of 0.5 ml of 1% formalin solution.	22	19	8	11
Fourth group of animals				
Implantation of tumor in the lateral ventricle of the brain (control).	22	18	0	0
Fifth group of animals				
Implantation of tumor in the testis.	16	16	15	63

However, our experiments show also that the property of tumors growing in the brain not to form metastases in internal organs is disturbed in case of application of an additional stimulus to an organ so far removed from the brain as the testis. Hence it can be concluded that on implantation in the tissue or ventricle of the brain and with subsequent survival and growth of the transplantate, the tumor cells enter the general circulation and reach the organs, but they do not survive and metastases are not formed. Tumor cells reaching the organs probably meet tissues resistant to them. This resistance is lost after injection of a small quantity of formalin into the testis.

The results obtained from our investigation not only confirm our previous observations but also demonstrate

the importance of central mechanisms both in the appearance and maintenance of resistance to the tumor cell, and also in its disappearance.

The absence of metastasis formation on implantation of the tumor in the brain, and its survival in that organ may be due to the inclusion in the response of the brain to trauma of protective and adaptative reactions which are not confined to the area of the brain itself but which also extend to the other organs and tissues of the body. The action of formalin on the testis caused interference with the mechanism of these reactions which probably led to those trophic changes determining the survival of the tumor cells circulating in the blood stream in a particular organ. This procedure also affected the growth of the tumor implanted in the lateral ventricle – which extended to the opposite lateral ventricle, thereby demonstrating trophic changes and loss of resistance in the brain itself.

SUMMARY

When Brown-Pearce tumor is implanted into the tissue of the brain or its lateral ventricle, the cells of the tumor, conveyed to internal organs, do not ingrow and no metastases are formed. Additional stimulation of the testis by injection of a small amount of formalin (after implantation of the tumor into the brain) resulted in formation of metastases in certain internal organs. Experiments prove the significance of the central nervous mechanisms in resistance of the tissues to tumor cells, as well as in disturbance of this resistance.

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CHANGES IN THE MYELOGRAM OF MICE WITH THE ASCITIC CARCINOMA OF EHRLICH

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There have been several reports by clinicians of investigations of the bone marrow in patients with carcinoma. These workers have attempted to find a "typical" myelogram of carcinoma, which could be used as a method of diagnosis and prognosis of carcinoma during life. The most pronounced changes in the blood and bone marrow are found in carcinoma of the stomach [1, 2].

In the peripheral blood in the majority of cases there is found a hypochromic anemia, neutrophilia with a shift to the left, and lymphopenia [1, 2, 8]. In the majority of patients there is a high leucocytosis; in some patients, on the other hand, leucopenia. However, the most important feature in this condition is presumably the decline in the hemopoietic and digestive function of the stomach, and disturbance of the metabolic processes in the patient affected by carcinoma is far less important. In carcinoma in other situations the morphological composition of the blood is changed to a smaller degree, or it may even remain within normal limits (carcinoma in an external situation).

In contrast to this, the bone marrow of patients with carcinoma always shows abnormal tendencies, irrespective of the site of the lesion. These abnormalities are most pronounced in carcinomatous lesions of the organs of the gastrointestinal tract, somewhat less so in lesions of other internal organs, and considerably less marked in carcinoma of the skin, tongue and breast. However, the myelogram is always abnormal – even in cases where the cellular composition of the blood of the patient remains normal.

Authors who have studied the myelogram of patients with carcinoma [1, 2, 4, 8, 9, 10] have found that it is not of the same type in all patients. In the bone marrow of the majority of them, for instance, there is found an increase in the promyelocytes, neutrophilic myelocytes and metamyelocytes. In some patients there is an increase in the number of adult neutrophils in the bone marrow [2]. The erythropoietic findings are contradictory: in some patients the number of erythroblasts is increased, and in others reduced [1, 3, 10, 9]. Some authors consider that a suppression of erythropoiesis is characteristic of carcinoma, and that the number of adult erythroblasts greatly exceeds that of the less mature [2, 8], while others consider that the number of erythroblasts sharply increases in carcinoma, and that there is a rise in the number of erythroblasts with basophilic protoplasm and in the number of proerythroblasts [4, 10]. According to the findings of all these workers the number of plasma cells in the bone marrow of patients with carcinoma is somewhat increased. O.V. Khakhaleva [9] considers that in malignant disease there is a most constant and characteristic hyperplasia of the reticular stroma of the bone marrow, while E.A. Fridman [8] found suppression of the reticular tissue of the bone marrow in the majority of patients with carcinoma.

From our point of view, such discrepancies can be explained by the variety of the clinical material. We were unable to find any experimental data on this subject in the literature accessible to us.

TABLE 1

Myelograms of Normal Mice

Animal No.	in percent															Number of nucleoli per 2000 cells
	Hemocyto-blasts	Myeloblasts	Proerythro-blasts	Erythro-blasts	Normo-blasts	Promyelo-cytes	Neutrophil myelocytes	Neutrophil metamyelo-cytes	Stab neutro-phils	Segmented neutrophils	Eosinophilic myelocytes	Eosinophils	Lymphocytes	Plasma cells	Megakaryo-cytes	Reticulo-cytes	Monocytes	
1	2.27	4.71	3.17	14.03	0.23	4.08	8.13	18.44	16.21	16.92	2.59	0.27	8.53	0.05	0.09	0.13	0.15	1
2	2.06	5.03	2.85	20.42	0.18	3.64	8.88	13.83	13.36	16.73	3.03	0.23	9.34	0.01	0.09	0.19	0.14	3
3	3.41	3.57	5.82	18.18	0.38	6.59	9.00	10.20	11.59	16.83	2.69	0.19	11.06	—	0.05	0.24	0.10	1
4	1.84	4.96	6.20	17.28	0.09	4.12	12.55	16.76	12.92	10.84	2.68	0.23	8.71	0.28	0.18	0.31	0.05	2
5	2.23	4.18	3.81	16.20	0.19	3.61	9.51	17.69	15.11	11.74	2.97	0.24	11.54	0.54	0.10	0.25	0.09	1
6	3.49	5.15	6.59	16.49	0.14	3.06	9.79	13.06	18.65	8.75	2.15	0.19	11.98	0.38	—	0.08	0.05	1
Average	2.55	4.60	4.74	17.10	0.20	4.18	9.64	15.00	14.64	13.63	2.68	0.23	10.19	0.21	0.09	0.20	0.10	1.5

The normal myelogram of the mouse has been described by workers studying mouse leukemia [3, 11, 12], but there is not yet the necessary unanimity on the names of the cells and on their interpretation.

The aim of the present investigation was to trace the changes in the myelogram in mice affected by the ascitic carcinoma of Ehrlich.

EXPERIMENTAL METHOD

The experiments were carried out on half-grown male white mice, weighing from 26 to 30 g. The experimental animals were injected intraperitoneally under sterile conditions with 0.2 ml of ascitic fluid taken from mice with the ascitic form of Ehrlich's carcinoma. The strain of ascitic carcinoma of Ehrlich had been kept in the Institute for a long time. The mice were then killed at intervals of one, three, five and seven days after injection of ascitic fluid. At each interval three mice were killed. The bone marrow was extracted from the right and left femora and two respective films prepared, fixed with anhydrous methyl alcohol and stained by the Romanowsky method. As a control six mice were killed and from their bone marrow films were prepared and stained by the same method as for the experimental animals. In producing the myelogram over 2000 bone marrow cells were counted in both films under oil immersion.

EXPERIMENTAL RESULTS

1. The Normal Myelogram of White Mice

We distinguished the following cells in the bone marrow of the mice: hemocytoblasts, myeloblasts, proerythroblasts, erythroblasts (basophilic and polychromatophilic together), normoblasts, promyelocytes, neutrophil myelocytes, neutrophil metamyelocytes (juvenile), stab neutrophils, segmented neutrophils, eosinophilic myelocytes, eosinophils, lymphocytes, plasma cells, megakaryocytes, reticular cells and monocytes. The normal average myelogram of the mice and its individual variations are given in Table 1.

We consider it necessary to give a brief morphological account of the features of certain cells and to describe granulopoiesis in mice.

Formation of nuclei of myelocytes in the bone marrow of mice may occur by the usual way or as a ring type. The great majority of myelocyte nuclei are formed in the latter type. A small, round hole forms in the center of the cell nucleus as a result of mitotic division. The chromosomes in the metaphase are distributed in the form of a ring with an empty space in the center. They are arranged in exactly the same way in the daughter asters in the telophase after separation of the chromosomes. In the later part of the telophase during formation of the nuclei, they

TABLE 2

Myelogram of Mice with Ehrlich's Ascitic Carcinoma

Time after inoculation	Animal No.	Hemocytoblasts	Myeloblasts	Proerythroblasts	Erythroblasts	Normoblasts	Promyelocytes	Neutrophil myelocytes	Neutrophil metamyelocytes	Stab neutrophils	Segmented neutrophils	Eosinophilic myelocytes	Eosinophils	Lymphocytes	Plasma cells	Megakaryocytes	Reticulocytes	Monocytes	Number of mitoses per 2000 cells
		In percent																	
1 day	1	5.40	5.04	3.60	20.08	0.09	3.88	13.98	15.53	11.75	9.28	4.02	0.61	5.21	0.23	0.14	0.23	0.33	9
	2	8.57	3.87	3.66	19.55	0.09	3.33	7.86	13.57	17.05	12.18	3.65	0.28	5.46	0.30	0.14	0.21	0.23	3
	3	5.66	6.39	3.01	12.59	0.09	3.21	20.17	15.09	13.44	9.62	3.82	0.09	6.18	0.13	0.05	0.10	0.37	5
3 days	4	8.51	4.19	3.72	8.17	0.09	5.03	11.27	12.71	15.76	18.37	1.15	0.09	10.15	0.29	0.09	0.16	0.25	4
	5	9.17	4.82	5.52	18.03	—	1.97	6.03	9.93	12.91	18.77	1.82	0.14	10.27	0.14	0.09	0.26	0.13	8
	6	9.87	5.09	4.54	17.94	0.19	2.39	6.81	10.26	13.78	13.59	1.26	0.09	13.25	0.19	0.09	0.30	0.36	4
5 days	7	8.88	5.04	4.81	15.87	—	3.25	3.98	10.95	11.29	15.25	3.20	0.28	16.15	0.71	—	0.15	0.19	6
	8	9.51	3.12	4.00	15.89	—	2.16	9.47	13.18	12.00	14.50	7.19	0.51	7.63	0.23	0.05	0.35	0.21	8
	9	11.67	4.17	2.64	8.21	—	4.81	6.96	13.69	15.95	21.57	2.05	0.09	6.77	0.57	0.10	0.25	0.50	7
7 days	10	7.33	4.53	3.21	9.04	—	5.11	6.31	12.44	17.17	25.55	1.22	0.05	6.19	0.28	0.09	1.2	0.28	8
	11	7.79	4.08	1.72	15.09	0.09	5.93	6.86	16.00	10.61	22.66	2.00	0.36	6.16	0.09	0.18	0.05	0.05	9
	12	8.58	4.62	3.68	8.58	0.04	4.98	10.81	14.09	19.03	16.01	1.64	0.22	6.76	0.44	0.09	0.14	0.15	6

TABLE 3

Summarized Average Myelograms of Mice with Ehrlich's Ascitic Carcinoma

| Group of animals | in percent | | |

the bone marrow of mice by A.Ia. Krashilina. We think that this group was really hemocytoblasts with irregularly-shaped nuclei. A.Ia. Krashilina herself writes that these cells do not resemble the monocytes of the blood.

In contrast to rats, typical basophils are found very rarely in the bone marrow of mice. In all the films examined we saw altogether one typical basophil myelocyte. The cells most often found had indistinctly outlined and washed-out basophilic granules, with a poorly contrasting nucleus. These cells found in the bone marrow of mice and rats are taken by some workers to be basophils [5]. In general, we do not think so. The cells of the erythroblastic series in mice are very clearly distinguished and no doubt arises about them.

2. Myelogram of Mice Affected by the Ascitic Carcinoma of Ehrlich

Until the fifth day from the moment of intraperitoneal injection of ascitic fluid into the mice, the circulation of peritoneal fluid was evidently unaffected. After the fifth day the development of ascites was observed, which must be due to interference with absorption of the peritoneal fluid. After the fifth day, and especially on the seventh day, wholesale death of the ascitic cells was observed. The ascitic fluid was translucent. After the seventh day the pressure of the peritoneal fluid was slightly raised, causing interference with respiration and the circulation of the blood. Two periods may thus be distinguished in the course of the disease. The first – until the seventh day, when all the symptoms are caused by proliferation of carcinoma cells and poisoning of the animal by products of their vital activities and death, and the second – after the seventh day, when the accumulation of a large quantity of fluid in the peritoneal cavity interferes with vitally important functions of the animal – respiration and circulation of the blood, which, in turn, may produce changes in other organs. For example, anoxemia arising as a result of disorder of the respiration and circulation may stimulate erythropoiesis in the bone marrow. However, this effect cannot be considered to be the direct consequence of the carcinoma. Accordingly we decided to investigate the bone marrow in the mice only in the first period of the disease, i.e., until the seventh day inclusive.

The myelogram of the animals at various intervals after injection of ascitic fluid and the mean values expressed as percentages are shown in Tables 2 and 3.

Comparing the mean values of the myelograms of the control and experimental animals (Table 3) it may be seen that the ascitic form of Ehrlich's carcinoma causes suppression of erythropoiesis in mice. This is shown by a gradual fall in the percentage of proerythroblasts and erythroblasts in the bone marrow. The most marked abnormal trend of erythropoiesis was observed in the later stages, i.e., on the seventh day.

The reaction of the cells of the neutrophil series appeared very quickly. In the earliest stages the percentage of immature forms increased at the expense of the mature, and next, conversely an uninterrupted rise in the segmented neutrophils was observed, with simultaneous reversion to normal of the relative metamyelocyte and myelocyte counts after their initial fall. The percentage of stab neutrophils changed only slightly throughout the experiment.

The indices of mitotic activity which we obtained from our investigation are only of relative importance on account of imperfections in the Romanowsky staining method. Nevertheless it can clearly be seen from Table 3 that the number of mitoses calculated per 2000 bone marrow cells after the first 24 hours of the Ehrlich's ascitic carcinoma is already three times greater than normal, and on the seventh day it is more than five times greater. Normally, the erythroblasts and neutrophil myelocytes are responsible for a large part of the mitoses, while in carcinoma it is the proerythroblasts and neutrophil myelocytes and metamyelocytes. Mitoses are met considerably more often among the hemocytoblasts than in the normal marrow.

In order to form a true opinion of the changes in hemopoiesis in carcinoma it was necessary to follow for the same periods of time the changes in the morphological composition of the peripheral blood. For this purpose we made repeated blood counts in four mice and then injected them intraperitoneally with 0.2 ml of ascitic fluid, then after one, three, five and seven days we took blood for analysis from the tip of the tail. The summarized mean results are shown in Table 4.

It can be seen from Table 4 that 24 hours after injection of the fluid the leucocyte count rose, on the third and fifth days it had returned to normal, and on the seventh day it again rose sharply. A moderate anemia was present by the third day. On the seventh day the number of erythrocytes per 1 mm^3 of blood fell to roughly 1,000,000. The leucocytic formula was altered: there was a gradual and significant increase in the percentage of neutrophils with a shift to the left; the percentage of juvenile forms in the blood rose; even myelocytes ap-

TABLE 4

Composition of the Blood of Mice with Ehrlich's Ascitic Sarcinoma. Summarized Mean Values

Time after inoculation	Date of taking blood	Erythrocytes per 1 mm^3	Leucocytes per 1 mm^3	Leucocytic formula						
				myelocytes	juvenile	stab neutrophils	segmented neutrophils	eosinophils	lymphocytes	monocytes
				in percentages						
1 day	6/8/1957	5,170,000	17,025	–	1.1	3.0	32.0	0.86	57.9	4.7
3 days	6/10/1957	4,570,000	10,125	0.4	0.9	3.1	34.0	1.9	51.8	5.5
5 days	6/12/1957	4,725,000	12,787	0.4	2.2	2.8	47.6	1.0	40.8	4.6
7 days	6/14/1957	4,072,500	22,100	0.1	2.7	4.0	53.3	0.6	35.4	3.4
Normal	6/1/1957 6/6/1957	5,397,500	12,918	–	1.2	3.1	17.0	0.87	68.3	9.0

peared, which are normally not present in the peripheral blood. At the same time the percentage of lymphocytes steadily fell. The percentage of monocytes was also considerably lower in carcinoma than in the normal state.

From a consideration of the changes in the peripheral blood and the simultaneous changes in the myelogram, it must be concluded that ascitic carcinoma in mice causes in the bone marrow some degree of suppression of erythropoiesis and a greatly increased production of neutrophil leucocytes.

The changes observed in the myelogram in mice with ascitic carcinoma are in general agreement with the findings of clinicians on bone marrow hemopoiesis in human carcinoma patients. However, there are differences. In the majority of clinical investigations cited above, for example, the neutrophilic reaction of the bone marrow was almost always characterized by predominance of immature over mature forms. In our experiments this picture was present only in the very beginning, and later the converse relationship held. It is possible that the cause of the difference lies in the different duration of the disease in man and animals and in the peculiarities of the ascitic form of carcinoma. In mice with carcinoma, except in the very earliest stages, erythropoiesis was always suppressed. In clinical observations the findings in this direction are most contradictory.

Attention should be directed to one more interesting fact which has not been found in clinical observations. In the myelograms of mice, starting on the first day after injection of ascitic fluid, the percentage of hemocytoblasts rose sharply. If in the normal myelogram the percentage of hemocytoblasts was 2.55, one day after the injection of ascitic fluid it was 6.54%, after three days – 9.18%, after five days – 10.02%, and after seven days – 7.90%. This reaction is all the more interesting because hemocytoblasts are the least differentiated cells in the bone marrow. In this case it is difficult to account for the reaction by changes in the hemopoietic processes alone, since the percentage of cells formed directly from hemocytoblasts (proerythroblasts, myeloblasts) is not increased.

SUMMARY

The cellular content of the bone marrow and the peripheral blood was studied in white mice in Ehrlich's ascitic carcinoma. Average progressive anemia and a gradually increasing neutrophilic leucocytosis was found in the blood. In 24 hours after the introduction of ascitic fluid the myelogram had already changed. Later the percentage of the hemocytoblasts was greatly increased while that of the proerythroblasts and erythroblasts in the bone marrow decreased. Production of neutrophilic leucocytes was much greater than in normal conditions and showed continuous increase. On the seventh day the mitotic activity of the bone marrow cells was five times greater than normal.

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THE ONCOLOGICAL CHARACTERISTICS OF THE CC-57 BROWN STRAIN OF MICE

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By way of preliminary remarks to our present paper, dealing with the history of the production of the CC-57 Brown strain of mice and their importance as subjects for biological and medical experiments, we may repeat the facts and arguments which appeared in our paper dealing with the CC-57 White strain of mice [5]. We shall therefore direct attention directly to the table in which are given the principal findings embracing the period of observation from February 12, 1952 to September 1, 1957.

As can be seen from the table in the current five-year period 233 mice were under observation. Some of these died a natural death and some were killed before reaching their maximum age.

Brown mice of the CC-57 strain, like the white, have at the present time passed through 35 generations from interbreeding of brothers and sisters, and have reached the same degree of homozygosis (99.99%) but are slightly less fertile than the White [5]. The duration of life of the brown mice may be judged partly from the data in the table, with, however, the correction that, as mentioned above, some of the mice were killed before reaching their maximum age.

From the oncological point of view the data given in the table merit scrutiny in the first place in respect to tumors of the mammary glands and lungs.

As we know, in mice of strain CC-57 Brown, from the first days of their existence (1943) until the end of 1952 carcinoma of the breast was not found [1-3]. V.I. Gel'shtein and A.M. Diad'kova [2] reported that they had found 15 tumors in this situation developing in brown mice during 1953-1954.

Analysis of the papers by these authors [2] on the basis of documentary data [4] showed that tumors in CC-57 Brown mice, described by the authors as being of that strain, in fact had no relation to it and belonged to mice of the so-called "herd" which we cannot dwell on here. In full accord with this are the new findings presented in this paper on brown mice which were and are being kept under observation by us. In fact, of the above-mentioned 233 mice, 112 females over seven months old died or were killed; carcinoma of the breast was not found in a single case. Thus CC-57 Brown mice like White are resistant to spontaneous tumors of the mammary gland, but being almost as susceptible to the milk factor, they are admirably suitable material for experimental investigation of problems of the etiology and pathogenesis of tumors of the breast.

Of no less interest is the problem of tumors of the lungs in CC-57 Brown mice. However, a detailed analysis of this problem is beset with the same difficulties caused by the mishandling of the statistical data in both the previous articles on the subject [1, 2]. As has already been pointed out earlier [4, 5], the statistical data on CC-57

Figures on the Number of Mice of Strain CC-57 Brown Dead or Killed and on Spontaneous Tumors Arising in Them (by age) During 1953-1957

Tumors	Sex of mice	Age of dead or killed mice in months																		Total
		5	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
	♀♀	9	15	7	21	3	9	6	7	14	15	2	17	12	—	4	—	1	1	143
	♂♂♂♂	8	8	5	14	5	2	14	6	8	4	1	8	6	1	—	—	—	—	90
	Total	17	23	12	35	8	11	20	13	22	19	3	25	18	1	4	—	1	1	233
		Appearance of tumors (in accordance with age)																		
Of the lungs	♀♀	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	♂♂♂♂	—	—	—	—	—	—	—	—	1 (1)	—	—	—	—	—	—	—	—	—	1 (1)
Carcinoma of the skin	♀♀	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1
	♂♂♂♂	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Leukemias	♀♀	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1
	♂♂♂♂	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Total	—	—	—	—	—	—	—	—	1	—	—	2	—	—	—	—	—	—	3

Note: The total number of tumors of the lungs is shown in parentheses.

mice in these articles were published in collective form, without subdivision into white and brown. For this reason, it was only possible to see from the published figures that tumors of the lungs do in fact arise in CC-57 mice, and quite impossible to establish what the true incidence of these tumors was in the brown and white mice separately.

In the course of the period under consideration here, when all the observations on the mice of this strain and the results of these observations were once again collected together in our hands, it was established that tumors of the lungs developed exclusively in CC-57 White mice and hardly ever appeared in Brown. The final results of our observations for five years are as follows: out of 182 white mice of an age for carcinoma in this situation, tumors of the lungs – for the most part multiple – developed in 15 (8.3%) [5], while of 107 brown mice of the same age only a single tumor of this organ was found (less than 1%).

When, in the course of observations of this strain, the difference between brown and white CC-57 mice was established with sufficient certainty, a special experiment was performed using urethane, which was injected into CC-57 White, CC-57 Brown and C-57 Black mice by M.B. Shimkin's method [7-9]. Mice of the last of these strains are known to be characterized by very high resistance to tumors of the lungs as indeed to all other tumors, and consequently may act as a safe control in such a series of experiments. The results of this experiment were as follows: the percentage of mice developing tumors after urethane treatment and the average number of tumors per mouse were found to be 100 and 7.18 for white mice, 15.8 and 0.15 for brown and 16.6 and 0.22 for black respectively. In accordance with international usage the brown and white CC-57 mice are given the symbols CC-57 BR and CC-57 W [6].

From these combined observations on mice of the various strains and on experimental CC-57 Brown mice there is every reason to assert that these are far more resistant to tumors of the lungs than mice of the related CC-57 White strain. It is possible also that the brown mice are more resistant to spontaneous tumors of certain other situations, and in particular to leukemia; further observations in this direction are proceeding.

Finally, as regards the incidence of tumors of the lungs in brown mice in the previous period, i.e., from 1943 until the end of 1952, as already pointed out above, this question was not satisfactorily dealt with in the published reports [1, 2] and must therefore be considered unanswered.

SUMMARY

The results of observations of the CC-57 BR strain mice are presented in this paper. They are now in their 36th inbred generation and reached nearly 100% of homozygosity. As in the preceding period of observations the CC-57 BR mice were found to be resistant to spontaneous mammary carcinoma and to spontaneous,as well as to induced tumors of the lung. However, they were sensitive to the milk factor and developed cancer in about 45 to 50% of cases.

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EXPERIMENTAL BIOLOGY

CELL DIVISION AND METABOLIC PROCESSES TAKING PLACE WITHIN THE ORGANISM

PART II. MITOTIC ACTIVITY AND BASAL METABOLISM

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In our previous paper [4] we described the results of a study of mitotic activity in relation to changes in blood carbohydrate content. Our findings gave definite evidence of the existence of a correlation between them. In view of this, and since carbohydrates are the substrates of oxidative processes in the tissues, we undertook the present experimental study with the object of ascertaining whether mitotic activity was dependent on the level of basal metabolism of the organism.

We reported in one of our earlier papers [5] that changing the environmental conditions of rats led to inhibition of mitotic activity, associated with considerable enhancement of gaseous metabolism. As these reactions faded out, i.e., as the animals became accustomed to their new surroundings, mitotic activity gradually rose to the normal levels, and oxygen uptake fell. These observations thus pointed to the existence of an inverse relationship between intensity of gaseous metabolism and mitotic activity. However, this antagonism, observed by us in relation to the reaction of the animals to environmental changes, may have been the result of the action of some third factor, functioning simultaneously, and participating in the achievement of the reaction. Such a factor might, for example, be adrenaline, the secretion of which by the adrenal glands is enhanced when the animals are in an excited state.

The question arose, in this connection, as to whether mitotic activity would be changed, and in what direction, in animals in which oxygen uptake was stabilized at a raised level, without involving change in environmental conditions. Does the intensity of cell division depend in any way on the basal metabolic rate?

EXPERIMENTAL METHOD

Our experiments were performed on male white mice which had been maintained for a number of days under standard conditions. We caused changes in their basal metabolism by applying the fully physiological procedure of segregating animals which had been members of a given group for a shorter or longer period, and placing them into separate cages. It is known from the work of a number of authors [1, 2, 3] that this causes a sustained rise in basal metabolism of certain mammals, including laboratory rodents. Since we had to study mitotic activity and oxygen uptake simultaneously, we conducted our experiments in gas-exchange chambers, specially constructed for the purpose (we used chambers of the type designed by N.I. Kalabukhov in our earlier experiments). The chambers were fitted with a device providing for the maintenance of an air-stream through them, and in this respect they did not differ from the usual respiratory exchange chambers; the only novel feature of our chambers was that they consisted of four separate chambers, in place of the usual single chamber, all four chambers working simultaneously. The mice were introduced into the respiratory chambers after they had been kept under standard conditions for some time. We measured respiratory exchange immediately after the

mice were placed in the chambers (they were then at once killed), and also after one and three days. In the latter two cases the air-tight roof of the chamber was replaced by wire netting when metabolic measurements were not being made, in order to ensure free access of air to the animals. The duration of the experiments involving measurement of oxygen intake was in all cases 30 minutes. The experiments were conducted during the fall and the winter, at room temperature (10-18°). Under such temperature conditions herd metabolic reactions are well developed [2, 3].

The oxygen intakes were calculated per kg live weight of the animal. Where the mice were kept in groups we calculated the oxygen intake per unit weight of the group taken as a whole. Mitotic activity was observed in fixed preparations of whole corneal epithelium. We took the mitotic index as being equal to the number of mitoses observed in 100 square fields of vision, corresponding to 1 mm^2 of corneal surface. We counted the number of mitoses, and recorded the numbers of each mitotic phase.

EXPERIMENTAL RESULTS

We performed 12 series of experiments on 96 animals. In six of the series we studied the correlation between mitotic activity and basal metabolism, while the other six served as controls of these six groups.

We killed eight mice of each of the first and second series, which resembled each other in every detail. Three mice, taken from the group of five, were distributed singly in separate chambers. The control group (five mice) remained together in a single chamber. All animals were killed immediately after completing measurement of respiratory exchange, i.e., 35-40 minutes after the members of the group had been segregated.

Respiratory exchange was measured in the third and fourth series after the mice had been kept in the respiratory chambers for some days.

In the fifth and sixth series the mice were kept in groups or singly for three days.

We performed control series of experiments, in order to assess the effect of the actual experiment of measuring gaseous metabolism on the mitotic activity of the groups of mice under investigation. In these series the animals were placed in the metabolic chambers as before, but respiratory exchange was not measured.

The mice of the seventh and eighth series were killed 35-40 minutes after having been placed in the respiratory chambers, after one day for the ninth and tenth series, and after three days for the eleventh and twelfth series.

The average results of mitotic counts for the corneal epithelium and of measurements of oxygen intake are presented in the table, covering all the series.

Our experiments gave very uniform results. Gaseous exchange was invariably greater in mice segregated singly for various lengths of time. Thus, if the mean oxygen intake per kg body weight was 756 ± 13 cm^3 (mean of all groups), the value found for mice kept in isolation for various lengths of time was 1403 ± 12 cm^3. The statistical significance of the differences between the respiratory exchange of the groups under comparison is such as to leave no doubt of the reality of the phenomenon.

As is evident from the numerical data presented in the table, the raised level of oxygen intake corresponds with a higher mitotic activity. In all 12 series the mitotic index of the corneal epithelium of isolated mice is much higher than for animals kept in groups. Statistical treatment of the data gives a sufficiently high degree of significance of the shifts in mitotic activity, both for each series separately and for all the series taken together.

Although the absolute values of the mean mitotic indices vary from experiment to experiment, the general shift in the number of mitoses observed in the corneas of solitary mice, as compared with the value found for those kept in groups, is found regularly. The column of figures representing mitotic activity of solitary mice differs considerably from that for mice kept in groups. The probability of this difference's being due to chance is less than 0.005.

The actual procedure of determining respiratory exchange had, in general, no appreciable effect on the significance of the observed differences between the experimental and the control series. The same shifts in mitotic activity were found in the blank experiments in which respiratory exchange was not measured as in those of the first six series.

Mitotic Activity of the Corneal Epithelium and Basal Metabolism of Mice Kept Singly or in Groups

Series No.	Duration of experiment	Animals kept together in a group		Animals segregated singly			
				mean mitotic index		mean oxygen intake (cm^3)	
		mean mitotic index	mean oxygen intake (cm^3)	in absolute figures	as % of control	in absolute figures	as % of control
1	40 min	130±13	736	161±7	124	1351±17	184
2	40 min	74±8	752	131±24	177	1396±56	186
3	24 hrs	113±14	805	247±60	219	1441±22	179
4	24 hrs	70±6	764	125±10	179	1396±6	183
5	72 hrs	122±7	782	159±5	130	1428±16	183
6	72 hrs	78±8	700	100±2	128	1404±42	201
7	40 min	40±5	–	115±43	287	–	–
8	40 min	52±11	–	113±10	217	–	–
9	24 hrs	109±5	–	145±5	134	–	–
10	24 hrs	146±10	–	187±11	128	–	–
11	72 hrs	83±13	–	176±20	212	–	–
12	72 hrs	103±10	–	161±5	156	–	–
	Mean	93±9	756±13	152±11	174±16	1403±12	186±3

We are thus fully justified in stating that all animals showing a sustained rise in basal metabolic rate are also distinguished by the high mitotic count of the corneal epithelium.

The figures representing relative changes in mitotic activity and in oxygen intake correspond fairly closely with each other: oxygen intake of solitary mice was raised by 86%, and mitotic index by 74%.

The observation that the mitotic count varies parallel with the basal metabolic rate after both long periods of isolation and after short ones, of the order of 35-40 minutes, suggests that not every relative increase in respiratory exchange taking place during a short period of time is necessarily associated with inhibition of mitosis of tissue cells, as might have been concluded from a study of the data of the previously cited paper [5].

The essential difference between our present experiments and our earlier ones is that we now transferred the animals of both the experimental and the control groups to the metabolic chambers. The reaction to their transfer to a novel environment should therefore have been the same in both groups of mice. It may hence be concluded that the inverse correlation between basal metabolism and mitotic activity is due to a reaction of the organism to novel environmental features. In those cases in which heightening of basal metabolism is not associated with this reaction it leads to an increase in the mitotic index.

We were not able to discern any significant or consistent differences between the relative proportions of the mitotic phases observed in the corneal epithelium of mice kept singly or in groups, the animals of the experimental and control groups being killed after having been kept in the metabolic chamber for the same lengths of time. This shows that the enhancement of mitotic activity of the corneal epithelium of animals having a high basal metabolic rate is the result of a more or less uniform and regular increase in the number of mitoses, affecting all the mitotic phases equally.

It would have been very desirable to compare the velocities of mitosis of the corneal epithelium of mice kept singly and in groups. Unfortunately, however, our attempts at assessing these velocities have not so far been successful, because of the experimental difficulties involved. Nevertheless, and irrespective of what may be the ultimate results of determination of velocities of mitosis at different basal metabolic rates, our results support the conclusion that the durations of mitosis and of the resting phase are in some way related to the basal metabolic rate. Intensification of metabolic processes, leading to a prolonged and sustained rise in the number of mitoses, determines the relative, and possibly also the absolute, shortening of the resting phase of the life cycle of the epithelial cells of the cornea.

This shortening of the resting phase appears to be due to intensification of synthetic processes in the cells.

SUMMARY

Data concerning the study of interrelationship between the mitotic activity of the corneal epithelium and the basal metabolic rate in mice are presented in this paper. Mice which were kept in individual cages and several mice kept together in one cage were used in this experiment. Oxygen consumption in mice kept in individual cages is permanently increased. The increased number of mitoses in the corneal epithelium corresponds to the rise in oxygen consumption by these animals. Increased number of mitoses is due to a more or less proportional increase of all stages of division. The authors came to the conclusion that the relative length of the interphase in the life cycle of a cell is decreased in animals with high basal metabolic rate.

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ORIGIN, DISTRIBUTION AND FUNCTION OF THE ACIDIC MUCOPOLYSACCHARIDES OF WHARTON'S JELLY IN THE HUMAN UMBILICAL CORD

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The umbilical cord is one of the more important organs of the embryo, the development and viability of which are largely determined on the condition of this organ. It is hence understandable that particular attention has been paid by research workers to the detailed study of its anatomical and histological features.

In the Russian literature, the fullest report of the histogenesis and microscopic structure of the umbilical cord and its Wharton's jelly is to be found in the papers published by M.Ia. Levina [6, 7, 8, 9]. A.V. Vikulov [2, 3, 4] has described an extravascular channel in the umbilical cord which seems to play some part in the development of certain diseases of the fetus during intra-uterine life, being a possible channel for the passage of infection from the maternal to the fetal circulation. This observation has obliged us to re-assess the function of the jelly of Wharton, which has heretofore been regarded as being only a protective covering for the umbilical vessels. It was of particular interest to institute a new approach to the study of the histogenesis of the umbilical cord and of Wharton's jelly, and, in particular, to the investigation of the origin, distribution, and functions of such physiologically active substances as are the acidic mucopolysaccharides, large amounts of which are deposited in the tissues of the umbilical cord.

EXPERIMENTAL METHOD

Umbilical cords taken from newborn babies and from fetuses (from the sixth to seventh week of development to full term) were fixed in 0.5% lead solution in 12% formalin. Paraffin sections were treated by the usual histological procedures. The acidic mucopolysaccharides were stained by means of histochemical methods, such as that of Hale, or the PAS reaction, as modified by McManus, Hotchkiss, and Gastaldi, staining with toluidine blue, with a parallel testicular hyaluronidase-treated control section for each of these procedures.

EXPERIMENTAL RESULTS

Umbilical tissue is little differentiated in the six–seven-week fetus. Wharton's jelly appears as a fibroblastic syncytium, still retaining certain mesenchymal features. The most characteristic feature of this stage of development is the copious secretion of jelly by the fibroblasts. The process of synthesis of mucopolysaccharides leads to accumulation of granules of mucoid substance within the cytoplasm of the fibroblasts, and these granules undergo displacement towards the surface layers of the cytoplasm, and are secreted by the cells. The processes of synthesis and secretion proceed in all the fibroblasts, but most actively in the cells of the perivascular zones. The fibroblasts are loaded with mucopolysaccharides in the form of granules of different sizes, from the smallest to grains the size of the nucleus, or even larger (Figure 1). The secreted granules absorb water to form a jelly-like substance in which filamentous structures subsequently make their appearance. The total amount of jelly is still small at this stage, however.

Fig. 1. Secretion of jelly-like substance by the fibroblasts of Wharton's jelly. Umbilical cord of a human embryo of 6-7 weeks development. Stained with toluidine blue. Objective Imm. 90. Gomal 6.

Fig. 2. Wharton's jelly of the umbilical cord of a newborn baby. Stained according to Hale. Objective x8. Gomal 6.

Rapid accumulation of mucoid substance takes place as a result of the process of active secretion, and this, in conjunction with accelerated differentiation of the tissues leads by the 9th-10th week to the formation of the umbilical vessels and of Wharton's jelly. Large bundles of fibers are formed, in particular in the subamniotic region. The characteristic appearance of Wharton's jelly now begins to appear: a reticular structure of bundles of fibers, in which are included elements of the fibroblastic syncytium and aggregations of the jelly-like substance. A considerable slackening off of secretory activity of the fibroblasts is seen at this stage. The falling off of synthesis and secretion of mucopolysaccharides is most marked in the central regions, which have been saturated with the jelly-like secretion. The secretory processes still remain fairly active in the peripheral parts of Wharton's jelly. The impression is received that the secretory process is of a zonal nature; side by side with groups of actively secreting fibroblasts can be seen zones giving no evidence of secretory activity. It is conceivable that this picture is the result of alternation of periods of secretory activity with periods of relative inactivity. At later stages of embryogenesis, and owing to the continuing differentiation of the fibroblasts, they progressively lose their powers of synthesis and secretion of mucopolysaccharides. Granules of mucopolysaccharides are still to be distinguished within their cytoplasm, but they do not attain the size seen at earlier stages.

Not until the 13th-14th week do separate mast cells appear in the tissues of Wharton's jelly. They arise from free, relatively undifferentiated cells located in the tunica adventitia of the umbilical vessels. We cannot exclude the possibility that they may originate from subendothelial elements of the umbilical vessels, but this possibility would require additional, very thorough verification. The mast cells synthesize and store granules of mucopolysaccharides. The number of mast cells increases, as a result not only of their new formation, but also of mitotic and amitotic division of already-formed cells, which migrate from the perivascular regions to the peripheral parts of Wharton's jelly, where they release their accumulation of mucopolysaccharide granules.

As the fibroblasts progressively lose their power of synthesis and secretion of mucoid substance, the function of production of mucopolysaccharides is taken over by the mast cells. Only a few scattered fibroblasts continue to produce mucopolysaccharides, up to the termination of pregnancy. Mucopolysaccharides are secreted almost exclusively by the mast cells during the latter months of pregnancy. The mast cells are not, however, fully able to compensate for the loss of the secretory function of the fibroblasts, and for this reason the relative concentration of acid mucopolysaccharides in Wharton's jelly falls perceptibly during the second half of pregnancy.

Wharton's jelly takes on a characteristic structure towards the end of pregnancy. It then takes on the form of a reticulum made up of well developed bundles of collagen fibers. Highly differentiated fibroblasts, retaining their syncytial connections, are to be seen, fused or trapped within the bundles. Very frequently, small amounts of mucopolysaccharides may be seen in the immediate vicinity of such fibroblast inclusions into the collagen bundles, affording evidence of the residual secretory activity of these fibroblasts. The meshes of the reticulum are filled with jelly, being a viscous aqueous solution of compounds of protein with acid mucopolysaccharides, of the type of hyaluronic or chondroitinsulfuric acid (Figure 2). The jelly is often traversed by strands of fibers.

The structure of Wharton's jelly varies greatly from one umbilical cord to another, as well as from one location to another in the same cord; the size and number of collagen fibers differ, as well as the size and shape of the meshes. The meshes filled with jelly, are in general stretched along the axis of the cord, and since they intercommunicate they constitute something of the nature of the continuous extravascular channel described by A.V. Vikulov. The meshes become particularly fine in the central perivascular zone, and between the vessels they appear in transverse sections as narrow slit-like spaces (Figure 3). The meshes become larger towards the periphery of the cord; the outermost subamniotic layer does not, however, usually contain mucopolysaccharides, but consists of a dense tissue, rich in collagen fibers. This subamniotic layer does not, as a rule, extend over the whole of the outer surface of the umbilical cord. In places, meshes containing jelly are situated immediately below the basal membranes of the amniotic epithelium.

Fig.3. Intervascular region of Wharton's jelly in the umbilical cord of a newborn baby. Stained according to Hale. Objective x8. Gomal 2.

We have as yet been unable to find a satisfactory explanation of the existence of such variations in the mutual relations of the jelly and the amniotic epithelium. In some cases the acid mucopolysaccharides fill only the peripheral meshes of Wharton's jelly, where they form considerable deposits. The meshes of the central parts of the cord contain a substance which, when treated by the ordinary procedures, resembles that of the peripheral zones, but which does not give a reaction for acid mucopolysaccharides. It might be supposed that this effect may be due to secretion and accumulation in the central regions of the cord of products of incomplete synthesis of mucopolysaccharide, but the explanation of this phenomenon remains obscure.

The conjunction of the thick bundles of collagen fibers with aggregations of jelly-like substance confers considerable strength and elasticity on Wharton's jelly, necessary for its undoubted role as a protective layer for the umbilical vessels. We believe, however, that this is not the sole, or even the most important function of Wharton's jelly. Papers published during the past few years have drawn attention to the importance of the biological protective function of substances of the type of the acid mucopolysaccharides, which are able to suppress the vital activities of a number of bacteria and viruses, and to inactivate their toxins [5, 10, 11, 12]. Evidence has been published [1] that secretion by the fibroblasts of Wharton's jelly is regulated by nervous impulses proceeding from the fetus.

A consideration of published data and of the results of our histochemical investigation of the umbilical cord, in conjunction with A.V. Vikulov's description of an extravascular channel, leads to a somewhat different approach to the evaluation of the functions of Wharton's jelly. According to Vikulov, the extravascular channel of the umbilical cord is made up of a system of intercommunicating cells filled with a "proteinic fluid" which flows towards the fetus. But this "proteinic fluid" is a very viscous jelly-like substance, and we think it highly improbable that it could circulate freely. The accumulation within the tissues of Wharton's jelly of enormous amounts of such biologically active material as are the acid mucopolysaccharides, the production of which is

probably regulated by the fetus, affords a basis for the assumption that Wharton's jelly functions as a kind of barrier to the spread of infection from the maternal to the fetal organisms.

SUMMARY

Acid mucopolysaccharides of Wharton's jelly were studied in umbilical cords of the newborns and in human embryos at various periods of pregnancy. The fibroblasts of Wharton's jelly synthesize and secrete acid mucopolysaccharides only during the first months of pregnancy (up to the fifth or sixth months). Mast cells begin to appear in the Wharton's jelly from the 13th-14th week and take over the function of synthesis and secretion of acid mucopolysaccharides. It may be assumed that the Wharton's jelly serves as a kind of protective barrier to the possible spread of infection from the mother to the fetus.

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MORPHOLOGY OF THE AMNIOTIC EPITHELIUM IN RELATION TO THE PROBLEM OF FORMATION AND RESORPTION OF AMNIOTIC FLUID

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Most of the studies of the amniotic epithelium have been related to the problem of the origin of the amniotic fluid. Recently published papers have described in considerable detail [7, 8, 10] the process of formation of vacuoles in the cytoplasm of the amniotic epithelial cells, thus confirming the original view [9, 11] that the amniotic fluid of humans and of some other mammals is of secretory origin. There is, however, evidence of the transudative origin of the fluid [1] in the human, from the chorionic vessels, based on the observation that vacuoles are rarely encountered in the epithelial cells earlier than the 16th week of gestation. In view of this contradictory evidence, we undertook the study of the amniotic epithelium of such animals as would, because of the morphological peculiarities of their membranes, present the most favorable conditions for transudation. We chose the allanto-amnion of swine, which has a vascular supply, and we studied it at the stage of development at which vascularization was at a maximum.

The vascular supply of the allanto-amnion of ruminants reaches maximum development about half way through the gestational period. It has been shown [3] that the vessels of the fetal membrane are situated closer to the amniotic surface. We considered that transudation of fluid from the blood vessels would be greatest at this stage of pregnancy. It was for this reason that we chose this stage for the study of the amniotic epithelium of swine allanto-amnion.

The boundaries of the amniotic epithelium appear in the form of festoons, and the thickness of the epithelial layer varies at different locations of the surface of the sac, in sections treated with silver nitrate according to Ranvier. This procedure regularly reveals nonnucleated cytoplasmic regions of various forms, which may possibly be sites of resorption of amniotic fluid. The presence of similar sectors in the epithelium of the other membranes [6] has been related to the process of resorption of amniotic fluid. The number and dimensions of such formations are highly variable (Figure 1). After short treatment (one to two minutes) with dilute (0.1-0.25%) solutions of silver nitrate, such sectors differ very little from the cytoplasm of the surrounding cells. With longer treatment, or using more concentrated solutions of silver nitrate, such nonnucleated sectors actively take up silver, and become stained brown or black.

Our attention was directed chiefly to the cytoplasm of the epithelial cells. Treatment with silver nitrate revealed the boundary between the endo- and the ectoplasm. We have been unable to find any mention of a similar effect in the epithelial or endothelial membranes. Silver is not deposited in the cytoplasm of all the epithelial cells. Where this does occur, the boundary between the endo- and the ectoplasm can be clearly distinguished. In sections stained with iron-hematoxylin the endoplasm is strongly basophilic, and is in clear contrast to the faintly-stained ectoplasmic zone. As will be shown later , it is in the endoplasm that continuous formation of vacuoles takes place, followed by their secretion into the amniotic sac. We believe that so clear-

Fig. 1. Nonnucleated cytoplasmic sectors of the amniotic epithelium of swine. One of the cells contains a clump of closely aggregated nuclei. Impregnated with silver nitrate, counterstained with iron hematoxylin. Photomicrograph. Objective x90, ocular x7.

cut a delineation of the endoplasm from the ectoplasm must in some way point to a difference between the roles of these structures in exchange processes affecting the amniotic fluid. Whereas the endoplasm is responsible for production and secretion of fluid, the ectoplasm would fulfill the opposite function of resorption of amniotic fluid.

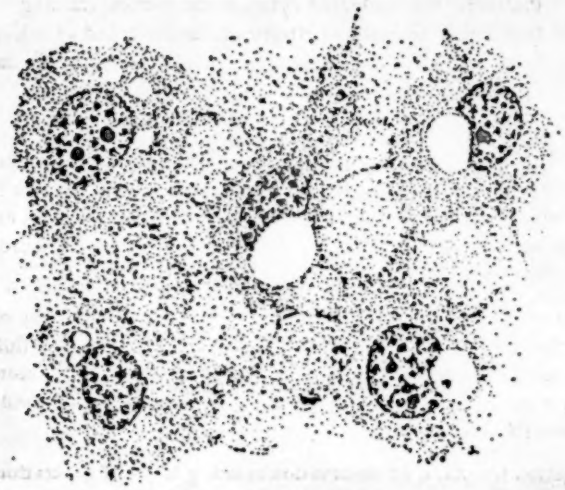

Fig. 2. Secretory vacuoles in swine amnion epithelium. Phase contrast. Objective x90, ocular x10.

The process of vacuolization of the epithelial cells is of particular interest. Vacuoles filled with a clear secretion are to be found in practically all the cells, in greater or smaller amount. Similar pictures have been

described by other authors, for the avascular human amnion [10]. In this case there can be no doubt as to the secretory nature of the vacuoles, and we attach particular importance to this morphological similarity.

The vacuoles begin to form in the perinuclear zone. We have been unable to confirm the observations of certain authors [10] who reported intranuclear formation of vacuoles; we could perceive them only in the cytoplasm, or more precisely, in the endoplasm of the cells. The earliest stage of their formation is the appearance of one or two clear droplets, in close contact with the nucleus. Their number rises progressively (Figure 2), and they run together to form large droplets, bulging into the lumen of the amniotic sac. All these processes take place in the perinuclear zone. The nuclei of some cells undergo deformation, due to the pressure of the secretion forming in the vacuoles. Giant cells, resembling fat cells, appear sometimes (Figure 3); their nucleus

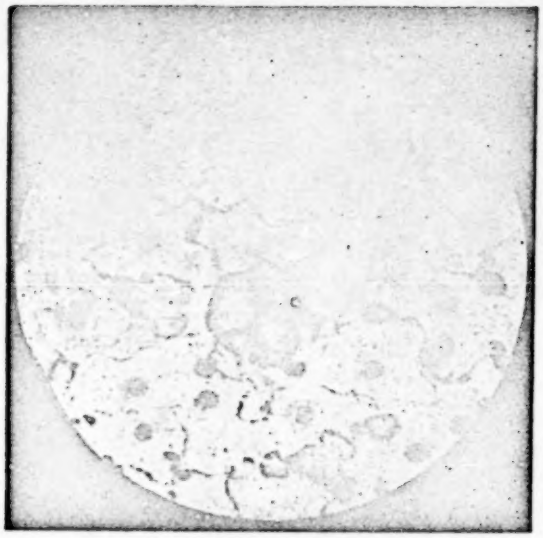

Fig. 3. Giant vacuoles in cells of swine amniotic epithelium. Nonnucleated cytoplasmic sectors, staining intensively with silver nitrate. Counterstained with iron hematoxylin. Photomicrograph. Objective x90, ocular x7.

or nuclei (these are more frequently large multinuclear cells) are squeezed out towards the periphery, and the whole of the cell is filled with the giant vacuole. We were unable to observe the formation of extracellular giant vacuoles, as reported by Bautzmann [8]. We invariably found nuclear matter, in one form or another, in the extracellular vacuoles. For this reason we consider that "extracellular vacuoles" should be regarded as being highly deformed uni- or multinuclear cells of the amniotic epithelium.

The conclusion may be drawn from our findings respecting the morphology of the amniotic epithelium of a highly vascular fetal sac that the secretory theory of the origin of the amniotic fluid is the most probable one. It is probable that production of amniotic fluid takes place as a result of the secretory activity of the amniotic epithelial cells in the early stages of gestation of the human and of animals in which blood vessels are absent from the membrane or from its vicinity.

During our investigation we made an observation relating to the regeneration of amniotic epithelium. It is known [4] that at an early stage of pregnancy the amniotic epithelium is regenerated by means of karyokinetic division. We observed isolated cases of karyokinesis at half-term in swine. Direct division of epithelial cells was even less frequently seen. At the same time, we more than once saw pictures closely resembling the end-stages of so-called endoamitosis. Something similar has been reported of recent years for human embryonic membranes [2, 5]. These authors reported the division of the large nucleus of the cells into a number of small nuclei, which

remained enclosed within the nuclear membrane for a certain time. In the terminal stages of this process the nuclear membrane disappears, but the daughter nuclei remain grouped together, so that each group of nuclei resembles a single giant nucleus of a cell. We have several times seen similar pictures, morphologically corresponding very closely with the terminal stage of endoamitosis, in the amniotic sac of swine (see Figure 1). While we record these observations, we by no means insist on the same explanation of their occurrence as has been proposed by the above authors [2, 5]. There can, however, be no doubt that what we saw in the amniotic epithelium very closely resembles the picture reported for endoamitosis. We would add, however, that the presence of endoamitotic division should in no way be considered to exclude the possibility of amitotic division or of karyokinesis.

SUMMARY

Considerable vacuolization of the epithelial cells of the amnion was found in swine during the stage of the greatest vascularization of the membrane. This supports the secretory theory of the origin of amniotic fluid. It is possible that resorption of amniotic fluid takes place through the cytoplasmic anuclear sites of various size at the area of the cellular boundaries.

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MORPHOLOGY AND PATHOMORPHOLOGY

CHANGES IN THE PERIPHERAL NERVOUS SYSTEM OF LABORATORY ANIMALS, ASSOCIATED WITH COOLING AND WITH ARTIFICIAL HYPOTHERMIA

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The application of artificial hypothermia to clinical medicine has been steadily increasing of recent years. Insufficient study has, however, been made of the functional and morphological effects of this procedure and of cooling the organism in general. There has been particularly little work done in this connection on the peripheral nervous system.

Cajal [9] has investigated the effect of cold on regeneration of nerves. He has also described changes taking place in motor ganglion cells of lizards during hibernation. Staemmler [16] has found degenerative changes in peripheral nerves following frostbite, and associated with peri- and endoneuritis. Legouix and Thieulin [10] have examined the effects of temperature fluctuations on peripheral nerves, and found that cooling lowered conductance of stimuli.

Müller [11] examined ganglion cells of the brain of cats in a state of artificial hypothermia induced by administration of Megaphen, and found severe degenerative changes, characterized by breakdown of Nissl substance, with formation of cytoplasmic vacuoles. The changes in the brain stem were of greater severity than in the cortex.

The object of the present study was to investigate morphological changes in peripheral nerves, appearing during hypothermia. We have been unable to trace any literature references to this subject.

Our experimental material consisted of 78 rats and 10 rabbits (64 experimental animals, and 24 controls). We examined the intramural cardiac ganglia, the intramural nerve plexi of the duodenum, and the richly innervated bursae of the knee and hip joints.

We lowered the body temperature of the first group of animals to 30°, 24° and 18°, by packing ice around them. The animals were maintained at these temperatures for three to six hours, or for 10-23 hours. The group consisted of 36 animals.

We induced hypothermia in the second group by the administration of Laborit's mixture (atropine sulfate 0.0005 g, phenobarbitol 0.055 g, isocaine 0.015 g, phenergen 0.025 g, tubocurarine 0.0001 g), at this dosage per kg body weight, and introduced over a period of two hours. The animals were then cooled to 30° or 24°, for a shorter or longer period, as in the experiments with direct cooling. We used 20 animals for these experiments.

In the third group of animals we produced neuroplegia by administration of Largactil. The body temperature fell to 33-34°, and the animals were maintained at this temperature for the same periods of time as in the other groups. This group consisted of eight animals.

Rectal temperature was measured every half hour. The animals were all killed in the same way, by administration of ether either immediately after the conclusion of the experiment, or one to three days later (10 animals).

The nerve elements were made visible by impregnation with silver, using Lavrent'evs modification of the Bielchowsky-Gross method [6], and, in addition, we applied Dogiel and Shabadash's procedure [15] of staining with methylene blue. These procedures, which stain nervous system elements in different ways, complement each other very satisfactorily. Material from the control aniamls was treated in the same way as those from the experimental groups. In order to achieve greater objectivity the slides were marked with code numbers.

EXPERIMENTAL RESULTS

We examined terminal nerve fibers, pericellular structures of vegetative intramural ganglia, and also receptors. The first degree of change perceptible in the structures was their heightened stainability, the next, irregularities in the thickness of the fibers (thickening or thinning), with formation of varicosities along their axes, which is considered in the literature to be evidence of an irritated state, preceding the onset of Wallerian degeneration.

Fig. 1. Arboriform receptor from special cells. Enhanced argyrophilicity and irregular thickness of terminal fibers after cooling to 24° for 5 hours. Articular bursa of a rat. Impregnated with silver.

In the normal animal larger or smaller unmyelinated fibers are distinguished by their smooth outlines and by their uniform thickness along the whole of their length. In the experimental animals, in particular in those which had been subjected to prolonged or profound cooling, we found, in addition to heightened intensity of staining, a thickening of the fibers, with formation of large varicosities.

The ganglion cells of the intramural cardiac nodes of the normal animal are surrounded by a network of regular fine pericellular fibers with smooth contours; these fibers become thicker after administration of Largactil. This manifestation of an irritated condition is particularly pronounced in cooled animals (Figure 1).

Evidence of irritation was also seen in the fibers of the nerve plexi of the conducting system of the heart of experimental animals.

The Auerbach plexus of the duodenum of normal animals is distinguished by the uniform thickness of the nerve fibers; in artificial hypothermia some of the fibers stain more intensely, their outlines become irregular, and varicosities appear.

The commonest type of nerve endings to be found in the articular bursae are the corpuscles of Ruffini, which originate from myelinated fibers by dichotomous ramification to give unmyelinated fibers distributed between intensely staining nuclei of special cells.

These terminal arborizations are more frequently encountered in artificial hypothermia because of their increased stainability. Neurofibrillar platelets could be seen at the ends of the unmyelinated terminal fibers of the arborizations, in specimens stained with methylene blue. Stimulation of these formations in cooled animals could be perceived in the form of thickening and uneven diameter of the ramifications of the unmyelinated fibers, or of varicose swellings (Figure 1).

Another type of encapsulated receptor commonly found in the articular bursae are the modified encapsulated corpuscles of the Vater-Paccini type (Golgi-Mazzoni bodies). The increase in their staining capacity was manifested not only by the increase in the number of stained corpuscles seen, but also by the more intense staining of the inner bulb of the corpuscle; we found 245 encapsulated corpuscles in 80 animals, and the number of such encapsulated receptors in animals subjected to artificial hypothermia was 2½ times as great as in normal animals, and in cooled animals four times as many were seen. The irritated state in cooled animals was manifested by thickening of the axon and by irregularities in its thickness (Figure 2).

Fig. 2. Encapsulated receptor of the Golgi-Mazzoni type. Enhanced argyrophilicity and irregularity of the axon after cooling to 24° for 5 hours. Articular bursa of a rat. Silver impregnated.

Fig. 3. Neuromuscular spindles. Enhanced argyrophilicity following cooling to 32° for 4 hours. Rat muscle. Silver impregnated.

We encountered considerable difficulties in staining the neuromuscular spindles of control animals. For this reason the greater staining capacity of the experimental animals is regarded by us as being a consequence of the cooling (Figure 3).

The enhancement of staining capacity and the signs of irritation of the nerve structures represent a nonspecific reaction of the peripheral nervous system to excessive stimulation, which may be produced by a great variety of factors. Such changes are described in the literature as being present throughout the parasympathetic system in pulmonary tuberculosis [7, 5], in hypertensive disease [4], and in oxygen hunger [2, 6]. We have also observed them under conditions of oxygen deprivation, excessive physical strain, artificially produced arthritis, and amputations [12, 13].

Similar changes are also to be found in normal animals, but to a much smaller degree.

Enhancement of staining capacity and formation of varicose swellings of the fibers have been described in the literature as being reversible phenomena. We could distinguish fewer fibers and receptors in animals killed one to three days after treatment than in those killed immediately after the conclusion of an experiment, which supports the view that the changes observed by us are reversible. It seems that metabolic changes occur in the cells, causing the increase in their capacity for staining with silver and methylene blue. It is logical to suppose that such changes in the fibers and receptors may lead to changes in conductance or receptivity of the structures. Wallerian degeneration, which is regarded as an irreversible condition, was not encountered in our experiments.

Changes in the peripheral nervous system resembling those described above have been reported by Astakhov [1], in his experiments on artificial hyperthermia. The changes due to cooling appear, however, to be more pronounced, and of longer duration.

Not all nerve fibers are equally affected. Our observations showed that thick terminal fibers proceeding from myelinated ones are the most affected, while the slender fibers accompanying the blood vessels remained unchanged. The experiments show that the sensory apparatus is less resistant than are the vegetative nerve fibers of the blood vessels, in which we could never observe any changes.

Our results show that the greatest changes in the peripheral nervous system are to be found in animals subjected to direct cooling. The effects are less pronounced (only heightening of staining capacity) in animals subjected to artificial hypothermia (by Laborit's procedure), and after administration of Largactil. These changes seem to be reversible.

The changes are greater the lower the body temperature of the experimental animals, and the longer they are maintained at low temperatures. The effects seen in the heart, the intestine, and the joint bursae are in general of the same kind.

SUMMARY

Experiments were performed on rats and rabbits. It was established that changes occur in the peripheral nervous system of the heart, intestine and the articular capsule in: 1) simple decrease of the body temperature by application of ice; 2) in artificial hypothermia induced by Laborits' method, and 3) in neuroplegia induced by largactyl. These changes are manifested by: a) increased staining; b) increased roughness of the nerve fibers and their varicosity. These changes mainly affect the thick terminal fibers and nerve endings, sensory function, while slender fibers of the vessels, vegetative by function, remain unchanged. The lower the body temperature in the animals and the longer the experiment, the more pronounced the changes in the nerve fibers. The most considerable changes appeared in the animals which were cooled by means of ice. In artificial hypothermia the changes were manifested mainly by increased staining of the nerve fibers and were reversible. Experiments show that hypothermia affects the whole organism. In the peripheral nervous system it mainly has an effect on the sensory nerves. However, these changes are not specific since they are also found in other prolonged interventions.

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HISTOPATHOLOGICAL CHANGES IN MUSCLES IN EXPERIMENTALLY PRODUCED OSTEOMYELITIS

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Published papers on osteomyelitis following gunshot wounds deal chiefly with the study of morphological changes in the muscles situated in the immediate vicinity of the inflammatory focus [11], and in the sciatic nerve [7]. Descriptions of morphological changes in the nervous apparatus of the muscles are to be found in only two case reports of this type of osteomyelitis [1].

In view of the general resemblance of this type of osteomyelitis to the experimentally produced form, we undertook the investigation of changes in the reactivity of the organism, including within this term also the morphological equivalents of reactivity, in experimental osteomyelitis proceeding in the presence of additional factors affecting the central and peripheral nervous systems.

Seven series of experiments were performed on 105 rabbits and 10 dogs.

EXPERIMENTAL METHOD

Osteomyelitis of the long bones of rabbits and dogs was produced by making an incision, 1-2 cm in length, under local anesthesia, at the medial surface of the left shin, and reaching to the bone. An opening 0.2 cm in diameter was then bored into the tibia, extending into the marrow cavity. We then introduced, through a cannula inserted into the borehole, 0.3 ml of a Staphylococcus aureus culture (250 million organisms per ml) per kg body weight of the animal.

In the first series of experiments the left sciatic nerve was divided and sutured, three to four weeks after appearance of osteomyelitis of the left tibia.

In the second series the left sciatic nerve was divided at a high level, after development of osteomyelitis symptoms, and vitamin B_1 was introduced into the peripheral stump, at a dosage level of 0.01 per kg body weight of the animal, after which the severed ends of the nerve were united.

In the third series the left sciatic nerve was not divided, but vitamin B_1 was injected into it at the same dosage.

In the fourth series we applied sleep therapy after the onset of osteomyelitis; this consisted in giving the animals subcutaneous injections of 2% Barbamyl (5 ml per kg body weight), twice daily for ten days.

In the fifth series we induced osteomyelitis ten days after division and repair of the left sciatic nerve.

The same was done in the sixth series, with the difference that sleep therapy was instituted simultaneously with induction of osteomyelitis.

The seventh (control) series consisted of animals with uncomplicated experimental osteomyelitis.

The experimental and control animals were killed three or six months after induction of osteomyelitis.

We made a histological examination of preparations of the gastrocnemius muscle, impregnated with silver by the Bielschowsky-Gross-Lavrent'ev procedure.

EXPERIMENTAL RESULTS

Histological examination of the left gastrocnemius muscle three months after operation of the animals of the first series showed atrophic changes in the muscle fibers, manifested by proliferation of the cell nuclei, with signs of irritation in the nerve structures, such as varicosities, excessive sinuosity, vacuolization, and irregular caliber of the nerve fibers. Deviations from the normal were not seen in the right gastrocnemius.

Six months after operation we found atrophic changes in the left gastrocnemius, with proliferation of muscle and connective tissue cell nuclei and large numbers of leucocytes, lymphocytes, and polyblasts were present in the muscle tissue; fairly marked evidence of irritation was seen in some nerve bundles and in individual axons, which were highly convoluted, of irregular caliber, often with clubbing, and coarsely impregnated with silver (Figure 1). Proliferation of muscle and connective tissue cell nuclei was seen in the right gastrocnemius, with coarse and incomplete impregnation with silver.

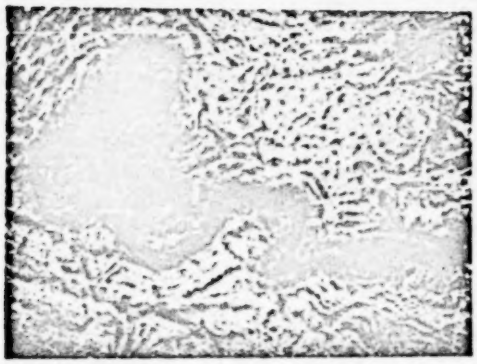

Fig. 1. Morphological changes in nerve structures of a dog's gastrocnemius, after induction of osteomyelitis, with subsequent division of the sciatic nerve. Excessive tortuosity of nerve bundles is seen 6 months after the operation. Impregnated according to Bielschowsky-Gross-Lavrent'ev.

In the second series of experiments we found atrophy of the muscle fibers, with proliferation of nuclei, three months after operation. In some of the sections perivascular round-cell infiltration, with an admixture of polyblasts, was seen. The nerve supply of the muscle was well developed, and large, medium, and small bundles of fibers were seen, consisting mostly of medium and small axons. Motor endings could not be seen. The nerve structures frequently showed signs of irritation, and were coarsely impregnated with silver. The right gastocnemius showed proliferation of muscle and connective tissue cell nuclei, and the nerve fibers were coarsely impregnated. Round-cell infiltration was seen sporadically.

Six months after the operation the left gastrocnemius showed increasing atrophy of muscle fibers, although the muscle was well supplied with nerves. The signs of irritation of the preterminal and terminal axons were less marked than at three months. Signs of irritation of the nerve fibers of the right gastrocnemius were evident, as well as proliferation of muscle and connective tissue cell nuclei.

Thus, the experiments of the first series showed fairly pronounced signs of irritation of the nerve fibers of the left gastrocnemius, which became more marked with the passage of time. These changes were more pronounced than in the second series of experiments.

The experiments of the third series showed that three months after the operation only coarse staining with silver could be seen in the nerve bundles, with only slight evidence of irritation of the preterminal and terminal axons of the left gastrocnemius. The motor nerve endings were clearly seen. The right gastrocnemius showed large foci of round-cell infiltration, chiefly around the blood vessels.

Atrophy of the fibers of the left gastrocnemius, with proliferation of muscle and connective cell nuclei, was seen six months after the operation. Motor nerve endings were visible. Signs of irritation were evident in some bundles of nerve fibers and in individual axons, such as irregular caliber, excessive convolution, edema-

Fig. 2. Morphological changes in nerve structures of a dog's gastrocnemius, after induction of osteomyelitis, with subsequent injection of vitamin B_1 into the intact sciatic nerve. An edematous condition of the neuroplasma of the preterminal axons is seen 6 months after the operation. Impregnated according to Bielschowsky-Gross-Lavrent'ev.

Fig. 3. Morphological changes in the nerve structures of a dog's gastrocnemius, after division of the sciatic nerve, followed by its repair. The preparation is from a dog with experimental osteomyelitis, and sleep therapy had been applied. A neuroma of a motor nerve ending had developed 3 months after the operation. Impregnated according to Bielschowsky-Gross-Lavrent'ev.

tous neuroplasm, and separation of fibers (Figure 2). Proliferation of muscle and connective tissue cell nuclei was seen in the right gastrocnemius, with only slight evidence of irritation of the preterminal axons.

Three months after the application of sleep therapy to the animals of the fourth series the preterminal axons of the left gastrocnemius showed only slight evidence of irritation. Proliferation of muscle and connective tissue cell nuclei was seen in the right gastrocnemius, and slight irritation of the nerve fibers was evident.

After six months only an insignificant degree of atrophy of the muscle fibers of the left gastrocnemius could be seen. No deviations from the normal were evident in the right gastrocnemius.

The experiments of the third and fourth series thus revealed a mild irritation of the nerve structures of both gastrocnemius muscles. Atrophy of muscle fibers was more pronounced in the later stages of the experiments of the third series.

Three months after the operation, and after induction of experimental osteomyelitis, acute atrophy of the muscle fibers was evident in the left gastrocnemius of animals of the fifth series, with increase in the number of muscle and connective tissue cell nuclei; the transverse striation of the muscle fibers could not be distinguished. Numerous streaks of fat were seen in some of the sections, with dilatation and thickening of the vessels. There were signs of acute irritation and fragmentation of the nerve structures of the muscle; excessive growth of nerve fibers was less frequently encountered. The nerve bundles consisted mainly of fine nerve fibers. Motor nerve endings could not be perceived. The nerve structures of the right gastrocnemius were coarsely impregnated, and the fibers were of uneven caliber. Preterminal and terminal axons sometimes formed loops.

Three months after operation of the animals of the sixth series muscle fiber atrophy was seen in the left gastrocnemius, with proliferation of muscle and connective tissue cell nuclei, and frequently with extensive foci of round cell infiltration. The blood vessels were very dilated, and had thickened walls. The nervous structures of the muscle showed signs of irritation and degeneration, such as varicosities, excessive convolution, edema of the neuroplasma, spiral fibers within bundles, neuromata of motor endings, and fragmentation (Figure 3). The right gastrocnemius showed coarse impregnation with silver, and proliferation of muscle and connective tissue cell nuclei.

After three months, the animals of the seventh series (controls) were found to have parts of the left gastrocnemius replaced by fatty tissue. Individual parts of the muscle showed granular degeneration. Extensive round-

cell infiltration was seen in some of the sections. Evidence of medium irritation and degeneration of nerve fibers was seen in the form of blurred contours and fragmentation. Proliferation of muscle and connective tissue cell nuclei was seen in the right gastrocnemius, while its nervous structures gave evidence of irritation and of low grade degenerative changes in the fibers, which were convoluted, of irregular caliber, twisted spirally, edematous, and fragmented.

After six months signs of inflammation were seen in the left gastrocnemius muscle, the nervous structures of which showed signs of irritation, and were only weakly impregnated. The only change found in the right gastrocnemius was in the coarse impregnation of its nervous structures with silver.

A comparison of the results of histological examination of the muscles of the animals of the first and second groups with those of the seventh (control) series shows that the signs of irritation of the nervous structures of the left gastrocnemius were more pronounced in the former two series than in the control series.

In the third and fourth series of experiments the signs of irritation of the nervous structures of this muscle were less marked than in the control series.

In the fifth and sixth series of experiments the signs of irritation and degeneration of the nervous structures of the left gastrocnemius were more pronounced than in the control series.

We consider that our observations confirm the view that there are two types of nerve regeneration processes; an adequate and an inadequate type [1, 5, 8].

The most severe forms of experimental osteomyelitis were found in the animals of the fifth and sixth series of experiments. Deep trophic ulceration of the heel developed sooner in these animals than in those of the first and second series, and they showed less tendency towards healing. X-ray examination showed that a considerable part of the tibia was affected by the destructive process. The histopathological changes in the gastrocnemius muscles and their nervous structures were also more acute. These findings suggest that preliminary division of the sciatic nerve, followed by its immediate repair, lowers the reactivity of the animals towards the pathogenic agent of osteomyelitis, and aggravates the subsequent pathological process; not even sleep therapy improved their condition.

Osteomyelitis took a much milder form in the animals of the third and fourth series of experiments. These animals showed no motor or trophic disturbances, the osteomyelitic process proceeded sluggishly, only small destructive lesions of the bone were seen, and only low-grade histopathological changes appeared in the muscle.

Introduction of vitamin B_1 into the intact nerve, and sleep therapy applied after infection both had a favorable effect on the animals, by creating conditions favorable for the strengthening of the compensatory capabilities of the organism.

The manifestations of a state of irritation of the nervous structures of the gastrocnemius muscles, observed by us, may be considered as being one of the manifestations of the compensatory action of the nervous system.

SUMMARY

This work was devoted to the study of histological changes in the gastrocnemius muscles and their nerve apparatus in experimentally induced osteomyelitis of the tibia. It was revealed in experiments with rabbits that a number of changes take place both on the affected side and in the healthy extremity. These changes are manifested in stimulation and degeneration of the nerve apparatus of the muscles which are more pronounced on the side of the lesion. Bilateral atrophies, deformations and inflammatory changes of the muscle fibers are observed. Changes on the healthy extremity are apparently of reflex origin. Excessive regenerative processes in the nerve apparatus of the muscles enhance dystrophic changes. Sleep induced by drugs and vitaminization of the nerves were applied in the third and fourth series of experiments. It was demonstrated that the change of the functional condition of the peripheral and the central portions of the nervous system increase the compensatory adaptation of the organism and decrease the tissue dystrophy. This evidently points to the reflex mechanism of the pathological changes described above and of the compensatory phenomena as well.

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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo(po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratorii	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoicz. Inst. Ekspt. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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